STUDY OF SERUM BETA-TRACE PROTEIN IN CHRONIC KIDNEY DISEASE

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DECLARATION

I, **Dr. R.SUSILA** hereby solemnly declare that the dissertation title "**STUDY OF SERUM BETA-TRACE PROTEIN IN CHRONIC KIDNEY DISEASE"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of the Department **Dr.N.Sasivathanam, M.D(Bio).,DGO.** This dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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CONTENTS

ABBREVIATIONS

CKD	: Chronic Kidney Disease
GFR	: Glomerular Filtration Rate
ESRD	: End Stage Renal Disease
CVD	: Cardiovascular Disease
LMWP	: Low Molecular Weight Proteins
BTP	: Beta-Trace Protein
Ccr	: Creatinine Clearance
IL-6	: Interleukin-6
eGFR	: Estimated Glomerular Filtration Rate
RAAS	: Renin Angiotensin Aldosterone System
KDIGO	: Kidney Disease Improving Global Outcome
PTH	: Parathyroid Hormone
TNF α	: Tumor Necrosis Factor α
USRDS	: United States Renal Data System
NSAIDS	: Non Steroidal Antiinflammatory Drugs
TGF-β	: Transforming Growth Factor-β

INTRODUCTION

Chronic Kidney Disease (CKD) encompasses a spectrum of different pathophysiologic processes associated with abnormal kidney function and a progressive decline in Glomerular Filtration Rate (GFR).[1]

CKD is defined as either kidney damage or a glomerular filtration rate (GFR) of less than 60mL/min/1.73 m^2 for 3 or more months" irrespective of the cause.[2]

CKD is a growing public health problem with increasing incidence and prevalence rates, poor outcomes, and high healthcare costs. Usually, CKD is a progressive disease leading to end-stage renal disease (ESRD), complications of decreased renal function, cardiovascular disease, and premature death.[3]

CKD is an international public health problem affecting about 5- 10% of the population and the expected incidence every year is approximately 5-8%[4]. It is an underestimate of the disease because most of the CKD patients die of CVD than to reach ESRD. CKD is the 12^{th} cause of death and 17^{th} cause of disability worldwide[5].

Risk factors and predictors for CKD progression include ethnicity, type of renal disease, and modifiable risk factors, such as blood pressure, proteinuria, smoking, dyslipidemia, obesity, and anemia, as well as exposure to nephrotoxins and baseline kidney function[6].

One of the prominent criteria for the diagnosis of CKD is decreased GFR value (< 60 mL/min/1.73m^2). GFR is widely accepted as the best index of kidney function. The normal value in young adult men and woman is approximately 125 mL/min/1.73m^2. Values below 15 mL/min/1.73m^2 indicate kidney failure and the person can be identified as a candidate for dialysis or renal replacement therapy/kidney transplantation[7].

Because of limitations of creatinine as a biomarker of GFR, new alternative biomarkers are being investigated, such as low molecular weight proteins. Different low-molecular-weight proteins (LMWP), with a molecular weight in the range 10–25 kDa, have renal handling compatible with that of an "ideal" marker of GFR[8].

In fact, they are cleared by the plasma through free glomerular filtration, subsequent complete tubular resorption, and degradation inside tubular cells. As a consequence, their serum concentrations increase progressively with the reduction of GFR. Furthermore, age, gender, and body composition have a low influence on serum concentrations of LMWP. Due to this behaviour, the measurement of serum concentrations of various LMWP has been proposed as a useful tool for evaluating an impairment of GFR, possibly more sensitive than serum creatinine[9].

Beta-trace protein (BTP) is a glycosylated LMWP, was primarily isolated as lipocalin-type prostaglandin D2 synthase from cerebrospinal fluid. It is freely filtered through the glomerular basement membrane and almost completely excreted via kidneys. Because of its low molecular mass, its constant production rate and its stability, BTP has been proposed as a new endogenous marker of glomerular filtration rate[10,11].

Hence in the present study, the serum levels of beta-trace protein is estimated in patients with different stages of CKD.

REVIEW OF LITERATURE

The kidneys play a central role in the homeostatic mechanisms of the human body, and reduced renal function strongly correlates with increasing morbidity and mortality.

KIDNEY

The kidneys form a paired organ system located in the retroperitoneal space. Kidneys receives approximately 25% of the cardiac output, about 90% of which supplies the renal cortex maintaining the highly active tubular cells. Nephron is the functional unit of kidney. Each kidney reported to contain between 6 lakhs and 1.5 million nephrons. Nephron consists of glomerulus, proximal tubule, loop of henle, distal tubule and collecting duct. Basic renal processes involved in urine formation are filtration, secretion and reabsorption[12].

FUNCTIONS OF KIDNEY[13]

1. Kidneys maintain output of water and array of electrolytes in close pace with their output and keep the body content of them nearly constant.

2. Kidneys accomplish tasks such as to excrete acids and bases to maintain balance and regulate the concentration of free hydrogen ions within a limited range.
3. Kidneys excrete end products of metabolic processes which serve no function and harmful at high concentrations. Kidneys along with the liver participate in removing toxic metabolites.
4. The crucial role played by kidneys in controlling blood pressure (BP) is direct or direct (by maintaining sodium and water balance) and by generation of vasoactive substances which regulate smooth muscle in the peripheral vasculature.
5. Kidneys secrete erythropoietin which is involved in control of red cell production.
6. Kidneys are responsible for the formation of active form of vitamin D.
7. During prolonged fast, substantial fraction of gluconeogenesis occurs in the kidneys particularly from non-carbohydrate sources (amino acids from proteins and glycerol from triglycerides).

CHRONIC KIDNEY DISEASE[14]

Chronic Kidney Disease refers to many clinical abnormalities that progressively worsen as kidney function declines. In 2002, The National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF-KDOQI) has proposed a definition for CKD to create uniform terminology to improve communication among patients, physician and researchers.

The definition of CKD is either

> "Kidney damage for ≥ 3 months, as defined by structural (or) functional abnormalities of the kidney with or without decreased GFR manifested by either Pathological abnormalities (or) markers of kidney damage including the abnormalities in the composition of the blood or urine or abnormalities in Imaging tests".
>
> (Or)
>
> "Glomerular filtration rate < 60ml/min/1.73 m^2 for ≥3 months with (or) without kidney damage."

To define CKD, the GFR should be below 60ml/min/1.73m^2 because it represents over a 50% reduction in kidney function as compared to the level for young healthy adults.

End Stage Renal Disease is defined as either GFR<15ml/min/1.73m^2 (or) a need to start Renal Replacement Therapy either in the form of dialysis

(or) renal transplantation. Most of the CKD patients will progress to ESRD and they require dialysis or kidney transplantation[15].

EPIDEMIOLOGY OF CKD IN WORLD[16]

CKD is a clinical syndrome that occurs as a gradual decline in renal function overtime. As per United States Renal Data System (USRDS) annual data report on 2007, one in nine adult has been affected with CKD and 20 million people are at risk for CKD. Increasing incidence may be due to aging population, Metabolic Syndrome, DM and an increase in the prevalence of Obesity. Of about 45% of Type 1 Diabetes mellitus patients develop progressive deterioration of kidney function within 15-20 years after diagnosis. Hypertension has profound effects on renal system. Obesity plays an important role in the development of kidney disease apart from its role as a risk factor for DM and HT.

GLOBAL PREVALENCE OF CHRONIC KIDNEY DISEASE[17]

- Incidence of CKD is increased two fold in the last 15 years globally.
- In the United States of America, 30million people suffer from CKD and 6 lakh people will require Renal Replacement Therapy.
- Over 1 million people worldwide are alive on dialysis.

- The reported global annual growth of number of ESRD patients is 7%[18].

CURRENT SCENARIO IN INDIA[19]

Approximate prevalence of CKD in Delhi is 7852 per million population and the incidence of ESRD is 785 per million (10% of total CKD.) DM has emerged as the most frequent cause (30-40%) followed by Hypertension (14-22%)[20].

STAGES OF CHRONIC KIDNEY DISEASE[16,21]

The National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF-KDOQI) proposed a widely accepted classification for CKD in which CKD is divided into 5 stages. This classification system is based on the level of estimated Glomerular Filtration Rate (eGFR). The higher the stage, the lower the GFR (or) vice versa.

This classification of staging provides the rough estimates of disease prevalence of different stages and the characteristics of individuals who are at increased risk or developing CKD (or) to allow the development of intervention plans for evaluation and management of each stage of CKD.

STAGES OF CKD	GFR (ml/min per $1.73m^2$)
0	>90[a]
1	≥90[b]
2	60-89
3	30-59
4	15-29
5	<15

'a' stands for associated risk factors of CKD. 'b' stands for demonstrated kidney damage (eg) Persistent proteinuria, Abnormal urinary sediment, Abnormal Blood and Urine chemistry or Abnormal Imaging studies.

GFR can be assessed by either 24 hours Urinary Creatinine Clearance or from serum Creatinine by using one of the following formulas[16,22,23]

1. **MODIFICATION IN DIET AND RENAL DISEASE(MDRD FORMULA)**

$$eGFR = 186 \times (Pcr)^{-1.154} \times (Age\ in\ years)^{-0.203}$$

- Multiply by 0.742 for women.
- Multiply by 1.21 for Blacks.
- Pcr – Plasma Creatinine in mg/dl.

2. **COCK CROFT – GAULT EQUATION**

$$\text{Estimated Creatinine Clearance} = \frac{(140\text{-Age}) \times \text{Wt in Kg}}{72 \times \text{Serum Creatinine}}$$

Multiply by 0.85 for females.

3. **CKD –EPI (EPIDEMIOLOGICAL COLLABORATION) FORMULA**

$$eGFR = 141 \times \min(SCr/k,1)^{\alpha} \times \max(SCr/k,1)^{-1.209} \times 0.993^{Age}$$

- multiply by 1.018 for females
- multiply by 1.159 for black
- SCr - serum creatinine (mg/dL)
- k is 0.7 for females and 0.9 for males
- α is -0.329 for females and -0.411 for males
- min indicates the minimum of SCr/k or 1
- max indicates the maximum of SCr/k or 1

NATURAL HISTORY OF CKD[24]:

Many patients with CKD, stages 3-5 progress relentlessly to ESRD. The relationship between the reciprocal of serum Creatinine values (1/Scr) or the estimated GFR and time is linear. A significant percentage of patients have breakpoints in their progression slopes leading to acceleration or slowing down of the rate of progression of CKD. They do not follow the predictable linear

fashion. The breakpoints due to lack of adequate control in systemic Blood Pressure or exposure to Nephrotoxins, Non Steroidal Anti-Inflammatory Drugs (NSAIDS) or Radio Contrast. The rate of progression of CKD varies depending upon the underlying pathology and the individuals. In diabetic individuals, the rate of progression of CKD is high. It is about 10ml/min/year reduction in GFR. In uncontrolled Hypertensive patients, it is about 5ml/min/year. If both DM and HT are controlled, the reduction in GFR is only 2ml/min/year.

In non diabetic individuals, the rate of progression of CKD is 2.5 times higher in chronic glomerulonephritis than in chronic interstitial nephritis and 1.5 times higher than in hypertensive nephrosclerosis.

ETIOLOGY OF CKD[25,26]

Location of Pathology	Systemic Diseases affecting the kidney	Primary kidney Diseases.
Glomerulus	Diabetes Mellitus, Auto Immune Disease, Systemic Infection, Drugs , Neoplasia.	Diffuse, focal, Crescentric and proliferative glomerulonephritis, focal and segmental glomerulosclerosis, minimal change Disease.
Tubulo Interstitium	Systemic infections, Auto immune Diseases, Sarcoidosis, environmental toxins, Urea and drugs	Obstruction (or) stones and Urinary infection.

Vascular Diseases	Atherosclerosis, Decreased perfusion (Liver disease, Heart failure, Renal artery disease), Hypertension, Vasculitis, Thrombotic microangiopathy.	ANCA (AntiNeutrophil Cytoplasmic Antibodies) associated vasculitis, Fibromuscular dysplasia.
Congenital	Alport Syndrome, Polycystic Kidney Disease, Oxalosis, Fabry disease.	Medullary cystic disease ,Renal dysplasia

PERCENTAGE OF PRIMARY DISEASE CAUSING CKD

Diseases	Percentage
Diabetic Nephropathy	31.2%
Undetermined	16.4%
Chronic Glomerulo nephritis (GN)	13.8%
Hypertensive Nephrosclerosis	12.8%
Others	11.7%
Tubulo Interstitial nephritis	7.0%
Obstructive Uropathy	3.4%
ADPKD	2.5%
Reno vascular disease	0.8%
Graft Dysfunction	0.3%
Total	100%

The above data are collected from the CKD registry of Indian Society of Nephrology Cumulative Report.

RISK FACTORS OF CKD[15]

NON MODIFIABLE RISK FACTORS

- Old age
- Race and ethnicity
- Gender
- Low birth weight
- Low socio-economic status

MODIFIABLE RISK FACTORS

- Obesity and Metabolic Syndrome
- Diabetes Mellitus
- Hypertension
- Uric Acid
- Proteinuria

MISCELLANEOUS FACTORS

- Smoking
- Alcoholism
- Caffeine
- Analgesic Abuse
- Dietary Phytoestrogens
- Lead and other heavy metal poisoning

CLINICAL PRESENTATION OF CKD[27]:

STAGE 1:

It represents kidney damage when GFR is normal or high. This includes patients with proteinuria (or) those with abnormal imaging studies.

STAGE 2:

- There is evidence of kidney damage with mild decrease in GFR.
- In both stages 1 and 2, patients are usually asymptomatic.
- Blood Urea Nitrogen and Serum Creatinine are normal.
- Acid Base, Fluid and Electrolyte balance are maintained by an adaptive increase of function in the remaining nephrons.

STAGE 3:

It includes patients with moderate decline in GFR. This is the stage where Serum Creatinine starts to rise. Majority of the patients still remain asymptomatic. Nocturia and polyuria are early symptoms that appear at this stage. Serum Creatinine and Blood urea nitrogen are increased and serum level of Erythropoietin, Calcitriol and Parathormone (PTH) are usually abnormal.

STAGE 4:

Patients present with severe fall in GFR, and overt Uremic symptoms like loss of appetite, nausea, anemia and recurrent infections. They also have hypocalcemia, acidosis, hyperphosphatemia and hyperkalemia.

STAGE 5:

When GFR < 15ml/min, there is worsening of all the aforementioned symptoms in these patients. At this stage patients require Renal Replacement Therapy.

FACTORS AFFECTING INITIATION AND PROGRESSION OF CHRONIC KIDNEY DISEASE[24]

INITIATION FACTORS

- **GENETIC PREDISPOSITION**

CKD often runs within families. Polymorphism of the gene encoding RAAS(Renin Angiotensin Aldosterone System), Nitric Oxide Synthase, Kallikrein, IL-1, TNFα , Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-β (TGF-β1), Plasminogen Activator Inhibitor-1(PAI-1), Complement factors and Immunoglobulins are the possible links of CKD.

- **RACIAL FACTORS**

Racial predisposition is attributed to a number of factors such as DM, HT Susceptibility and also genetic susceptibility . Social deprivation or low social economic status are linked to the higher prevalence of CKD in the developing countries.

- **MATERNAL AND FETAL FACTORS**

Maternal malnutrition during pregnancy and resulting fetal malnutrition may contribute to the development of HT, Metabolic Syndrome, DM, CKD in adult life. Reduction in the number of nephron at birth (oligonephronia) and their ability to handle increased solute and salt load leads to glomerulosclerosis and CKD.

- **OTHER FACTORS**

Males and elderly people are more prone to develop CKD.

INITIATION MARKERS

- Hypertension – Elevated BP in both men and women is a risk factor for ESRD.
- Diabetes Mellitus
- Hyperlipidemia – Increased Triglycerides(TGL) is associated with CKD.
- Obesity
- Smoking is associated with increased risk for proteinuria in men.

PROGRESSION FACTORS

The progression of CKD is variable and associated with the variety of risk factors and markers:

AGE

Rate of progression of CKD is influenced by age. Elderly Patients affected by GN are having a faster rate of GFR decline than young people except Type1 DM, in which young individuals are having a faster rate of GFR decline.

GENDER:

Male gender are often associated with more rapid GFR decline and rapid progression.

RACE:

In United Kingdom, Indo-Asian patients with Diabetic Nephropathy have a faster rate of decline of GFR than Caucasians.

GENETICS:

Patients with Polycystic Kidney Disease (PKD) carrying the genotype PKD1, have a worse prognosis than others. Angiotensin Converting Enzyme (ACE) gene polymorphism, either deletion (or) Insertion also involved in linking between susceptibility and progression of CKD.

LOSS OF RENAL MASS:

The threshold for nature progression in terms of GFR loss appears to be crossed when loss of nephron function exceeds 50%.

MODIFIABLE RISK FACTORS AND MARKERS:

HYPERTENSION

Transmission of systemic hypertension into glomerular capillaries and the subsequent development of glomerular hypertension contributes to the initiation and progression of glomerular sclerosis.

PROTEINURIA

Degree of proteinuria is associated with the rate of progression of CKD. Heavy proteinuria is associated with faster rate of progression. Non selective proteinuria is mainly responsible for the natural progression of CKD whereas the highly selective proteinuria (eg) Albuminuria can persist for more than 10 years in the nephrotic range without causing structural damage to the kidney.

METABOLIC MARKERS AND FACTORS

- **GLYCEMIC STATUS**

  Degree of glycemia is associated with the rate of progression of CKD. Higher the degree of glycemia , faster the rate of progression of CKD.

- **LIPIDS**

Dyslipidemia is the contributory factor for glomerulosclerosis and tubulointerstitial fibrosis.

- **OBESITY**

Excess body weight and high body mass index have been linked to a rapid progression of CKD.

- **URIC ACID**

Hyperuricemia may cause hypertension and renal injury through stimulation of Renin Angiotensin System.

MISCELLANEOUS FACTORS

- **SMOKING**

Cigarette smoking increases systemic blood pressure and alters the renal hemodynamics leading to rapid progression of CKD.

- **ALCOHOL AND RECREATIONAL DRUGS**

Alcohol consumption increases the rate of progression of CKD through the effect of hypertension. The use of recreational drugs such as Opiates is associated with the progression of CKD.

- **CAFFEINE**

  Excessive exposure to Caffeine leads to progression of renal scarring.

- **ANALGESICS AND NSAIDS**

  Ingestion of Phenacetin, Paracetamol, Aspirin and NSAIDS are associated with the increased risk of ESRD.

- **LEAD EXPOSURE:**

  Chronic Lead exposure is implicated in the development of ESRD.

PATHOPHYSIOLOGY[28]

The pathophysiology of CKD is a complex process and is dependent on the primary cause. After an acute or chronic insult, many common pathways are activated to perpetuate glomerular and tubulointestinal injury. There are two types of injuries.

1. Hemodynamic injury
2. Non-hemodynamic injury

HEMODYNAMIC INJURY

This process occurs at a linear rate in proportion to the greater reduction in kidney mass resulting in an increase in renal plasma flow and hyperfilteration of the remaining nephrons. Systemic hypertension and RAAS mediated glomerular hypertension cause progressive glomerular damage and proteinuria, resulting in decreased afferent arteriolar tone than the efferent tone. This net efferent vasoconstriction increases intraglomerular pressure and filteration pressure further more, perpetuating hyperfilteration injury.

With loss of functioning nephrons, Renin is released from the Juxta Glomerular Apparatus due to decreased perfusion pressure and low Sodium delivery to the Macula Densa. Renin converts Angiotensinogen to Angiotensin I. Angiotensin I is converted into Angiotensin II with the help of Angiotensin Converting Enzyme. Angiotensin II is the main perpetrator of glomerular hemodynamic maladaptation.

Angiotensin II is the potent vasoconstrictor in the post glomerular arterioles. It also increases proximal tubular Sodium reabsorption directly and distal tubular Sodium reabsorption indirectly through Aldosterone. Lastly, it also stimulates posterior pituitary to release AntiDiuretic Hormone.

All these mechanisms are an integral component of autoregulation helping to maintain GFR when perfusion is decreased. The increase in glomerular hypertension increases the filteration fraction and radius of the pores in the glomerular basement membrane resulting in clinical proteinuria and glomerular destruction.

NON-HEMODYNAMIC INJURY

Non hemodynamic maladaptive pathways lead to inflammation and fibrosis of kidney. Angiotensin II level is increased in virtually every compartment of the kidney such as mesangial cell, endothelial cells, podocytes, the urinary space (Bowman's capsule) and the tubulointerstitium.

Increased Angiotensin II which upregulates several growth factors and their receptors like Connective Tissue Growth Factor, Epidermal Growth Factor, Insulin Like Growth Factor-1, PDGF, Vascular Endothelial Growth Factor(VEGF), Transforming Growth Factor-β and Monocyte Chemotactic Protein -1. The activation of these factors leads to over production of extracellular matrix by upregulating other factors such as Type1 Procollagen, PAI-1 and Fibronectin. In addition to that, excess adhesion molecules like

Integrins or Vascular Cell Adhesion Molecule 1 allow the increased extracellular matrix and hypercellularity to accumulate resulting in cell proliferation, extra cellular matrix accumulation, adhesion of these cells and functional changes ultimately resulting in fibrosis.

Inflammation is the key factor in the progression of all types of kidney disease and it is mediated partly by RAAS. Angiotensin II recruits macrophages and T cells by stimulating Endothelin -1 and increased the production of Nuclear Factor k light chain enhancer of activated B cells. These molecules will release cytokines creating more inflammation. TGF-β is also responsible for cellular recruitment. Free radical oxygen species creates an additional injury which enables further inflammation and fibrosis.

Through primary stimulation of the RAAS, a cascade of events beginning with inflammation occurs which is perpetuated by accumulation of cells and matrix, and is exacerbated by adhesion of these cells and matrix resulting in glomerulosclerosis and tubulointerstitial necrosis. This creates a progressive course of CKD, proteinuria, decline in GFR and a vicious cycle of continuous RAAS activation.

MECHANISMS OF PROGRESSION OF CHRONIC KIDNEY DISEASE[24]

The progression of CKD is associated with the progressive sclerosis of glomeruli irrespective of the nature of underlying nephropathy. Both intra and extra glomerular cells contribute to the initiation and progression of glomerulosclerosis.

INTRA GLOMERULAR CELLS

ROLE OF GLOMERULAR ENDOTHELIAL CELLS:

Glomerular endothelium performs an important role in preserving the integrity of vascular beds of glomeruli. They are the first exposed to the damage caused by hemodynamic injury, immunologic and metabolic injury. This endothelial injury is associated with the loss of their anticoagulant and anti inflammatory characteristics and gain of procoagulant and inflammatory properties leading to attraction and activation of platelets and microthrombus formation.

It is further associated with the initiation of glomerular micro inflammation with the attraction, adhesion and infilteration of glomerular tufts by monocytes. Then platelets and monocytes interact with mesangial cells resulting in production of extra cellular matrix (ECM).

ROLE OF MESANGIUM

After endothelial injury, the infiltrating monocytes interact with the mesangial cells and stimulate them either through direct cell to cell interaction or through release of mitogens like PDGF. The transcription factor kappa B (NF-κB) and a variety of kinases [Mitogen Activated Protein kinase (MAPK) and Jun N-terminal kinase or stress activated protein kinase] are involved in the proliferation of mesangial cells.

Activated mesangial cells have the capacity to revert to a myofibroblasts expressing markers such as α smooth muscle Actin, under the control of Fibrogenic Growth Factor like TGF-β1 and synthesizes interstitial Type III collagen which is not a normal component of glomerular extra cellular matrix. Resolution of glomerular and mesangial sclerosis depends upon the balance between the increased extra cellular matrix and its breakdown by metalloproteinases and glomerular collagenases.

ROLE OF GLOMERULAR EPITHELIAL CELLS

The relative inability of podocytes to replicate with respect to injury may cause their stretching along the glomerular basement membrane. This will expose the areas of denuded glomerular basement membrane. Attraction and interaction of denuded glomerular basement membrane with the parietal epithelial cells forms capsular adhesions and subsequent segmental

glomerulosclerosis. Tuft-to-capsule adhesions allow the influx of periglomerular fibroblasts into the glomerular tuft causing glomerulosclerosis.

EXTRA GLOMERULAR CELLS:

PLATELETS

The stimulation of the coagulation cascade by the activation of platelets and their release products will activate the mesangial cell and promote its sclerosis.

- Thrombin stimulates TGF-β1, resulting in progression of mesangial extra cellular matrix production.
- Upregulation of Plasminogen Activator Inhibitor-1 within the damaged glomeruli may lead to extra cellular matrix accumulation and glomerulosclerosis because of its inhibition of proteolytic enzyme plasmin.

Degree of glomerulosclerosis depends upon the balance between thrombotic- antiproteolytic and anticoagulant- proteolytic activities.

LYMPHOCYTES, MONOCYTE AND MACROPHAGES

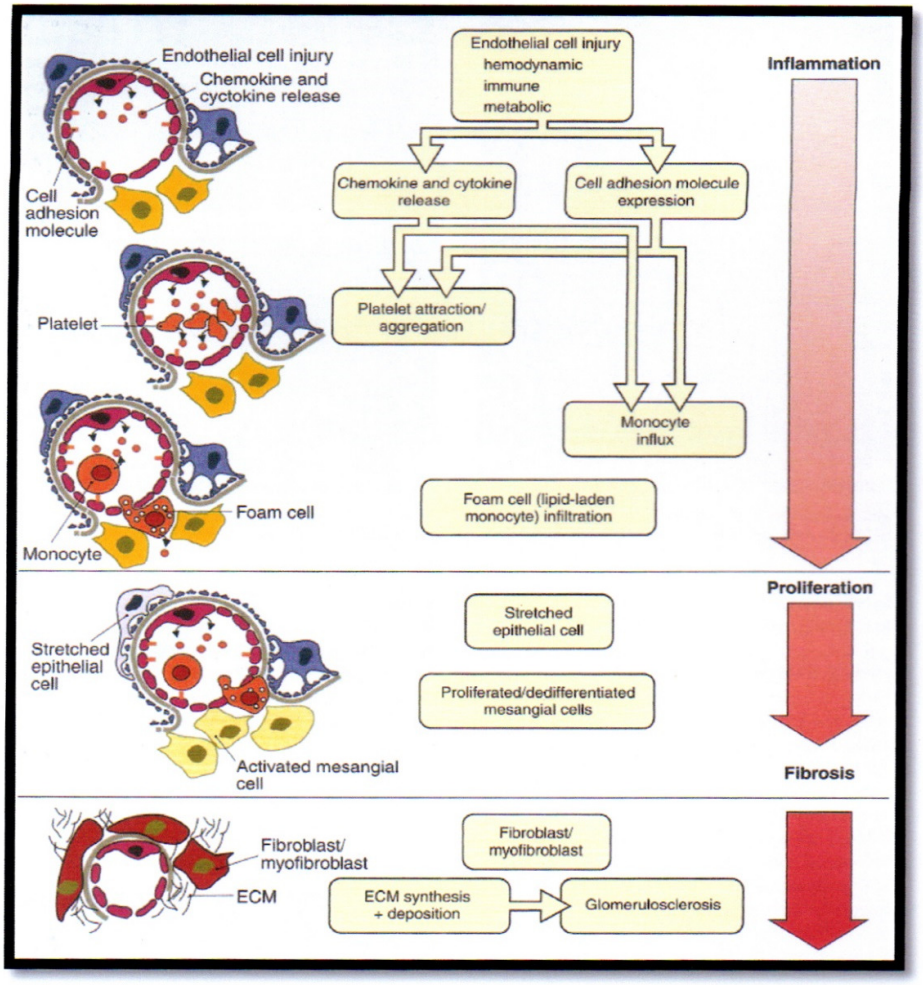
The release of Cytokines, Growth Factors and Procoagulant factors by lymphocytes as well as monocytes and macrophages is likely to contribute to the pathogenesis and progression of glomerulosclerosis.

BONE MARROW – DERIVED CELLS

Hematopoietic stem cells are involved in the normal glomerular cell turnover and response of glomeruli to injury.

FIGURE 1

STAGE OF GLOMERULOSCLEROSIS



TUBULO INTERSTITIAL SCARRING:

Tubulo interstitial fibrosis is developed in three stages.

1. Inflammation of tubulo interstitium.
2. Proliferation of interstitial fibroblasts.
3. Excessive deposition of interstitial extracellular matrix

Renal tubular cells play an important role in the pathogenesis of tubulointerstitial fibrosis. Injured tubular cells act as antigen presenting cells expresses cell adhesion molecules and releases inflammatory mediators and growth factors resulting in increased synthesis of ECM.

Loss of complementary proteins in the glomerular proteinuria may damage the tubular cells. Tubular cells may also be stimulated by the spill over of hormones such as Angiotensin II, Growth Factors and Cytokines from injured glomeruli.

Activation of tubular cells and their release of chemotactic factors can attract inflammatory cells including monocytes to the tubules and renal interstitium with subsequent activation of renal fibroblasts.

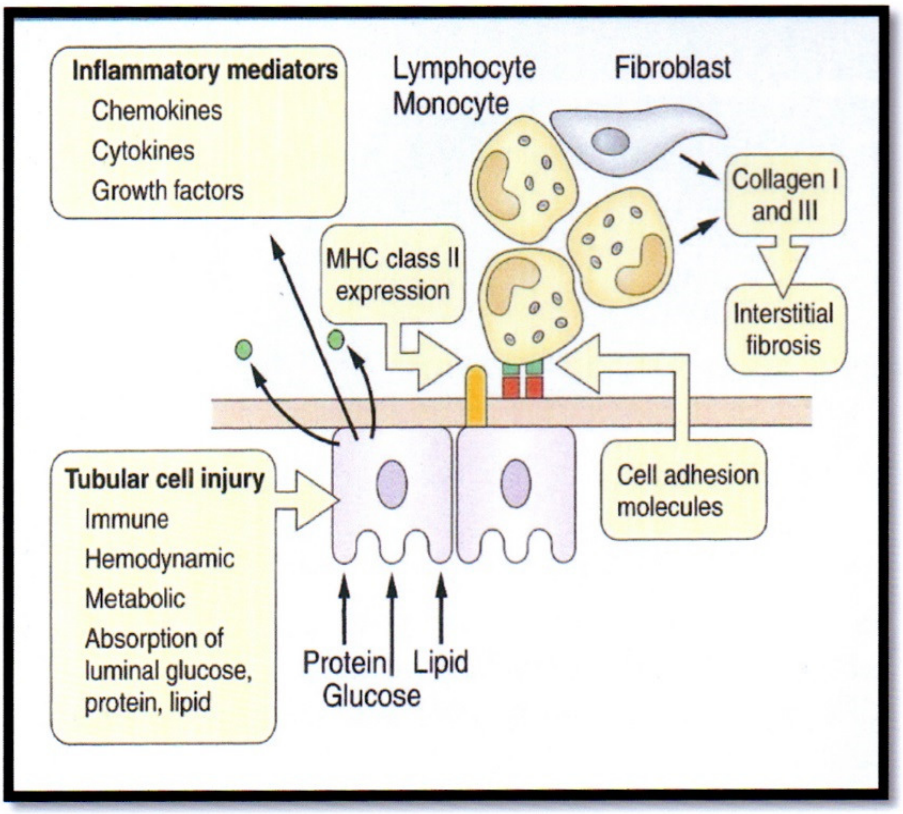
Activated renal fibroblasts acquire myofibroblast characteristics [eg. express α – smooth muscle Actin and synthesize interstitial Type I and III collagen] proliferate and invade the periglomorular and peritubular spaces. The resolution of deposited extracellular matrix depends on activation of Matrix Metalloproteinases and Plasmin. Inhibition of these two protolytic enzymes results in tubulo interstitial scarring.

Tubular cells contribute to fibrogenesis through their transformation into a myofibroblastic phenotype is called epithelial mesenchymal transformation.

This is a form of reverse embryogenesis because proximal tubules are derived ontogenetically from the metanephric mesenchyme.

FIGURE 2

STAGE OF TUBULOINTERSTITIAL FIBROSIS



VASCULAR SCLEROSIS

This is an integral feature of the renal scarring process. Renal arteriolar hyalinosis is associated with progression of CKD. This may be present at the early stage of CKD, even in the absence of severe hypertension. Hyalinosis of afferent arterioles may be implicated in the pathogenesis of glomerulosclerosis. Hyalinosis of the post glomerular arterioles may exacerbate interstitial ischemia and fibrosis. Ischemia and the ensuing hypoxia stimulate tubular cells and kidney fibroblasts to produce extra cellular matrix components and reduce their collagenolytic activity.

COMPLICATIONS OF CKD

ANEMIA[29]

Several factors are implicated in the development of anemia.

They are

- Erythropoietin deficiency.
- Retention of Bone marrow toxins .
- Bone marrow fibrosis secondary to hyperparathyroidism.
- Haematinic deficiency – Iron, Vitamin B_{12}, Folate.
- Increased red cell destruction.
- Abnormal red cell membranes causing increased osmotic fragility.
- Increased blood loss – occult gastro intestinal bleeding, blood loss during haemodialysis or because of platelet dysfunction.

- Drugs like ACE inhibitors may cause anaemia in CKD by interfering with the control of endogenous Erythropoietin release[30].

CKD-Mineral and bone disorder (CKD-MBD)[31]

A systemic disorder of mineral and bone metabolism due to CKD is manifested by either one or a combination of the following:

- Abnormalities of Calcium, Phosphorous, Parathormone and vitamin D metabolism.
- Abnormalities in bone turn over, mineralization volume, linear growth or strength.
- Vascular or other soft tissue calcification.

The term Renal osteodystrophy should be used exclusively to define alterations in bone morphology, following bone biopsy, associated with CKD.

- As GFR declines, plasma phosphate concentration rises, resulting in reduced ionized calcium. The consequence of this is increased production of parathyroid hormone (PTH) by the parathyroid glands.
- Fibroblast growth factor-23 (FGF-23) is part of a family of phosphatonins that promotes renal phosphate excretion. FGF-23 concentrations increase markedly in CKD which may also stimulate PTH.

- Decreased renal production of the 1α- hydroxylase enzyme results in reduced conversion of 25-OH cholecalciferol to 1,25 dihydroxy cholecalciferol.
- Reduced activation of vitamin D receptors in the parathyroid gland leads to increased release of PTH.
- Calcium sensing receptors expressed in the parathyroid glands react rapidly to acute changes in serum Calcium concentrations and a low Calcium also leads to increased release of PTH.
- 1,25 dihydroxy cholecalciferol deficiency also results in gut Calcium malabsorption.
- The result of these complex metabolic disturbances is secondary hyperparathyroidism.

- Increased PTH secretion promotes reabsorption of Calcium from bone and increased proximal renal tubular reabsorption of Calcium and this opposes the tendency to develop hypocalcemia induced by 1,25 dihydroxy cholecalciferol deficiency and phosphate retention[32].

- **FLUID, ELECTROLYTE AND ACID-BASE DISORDERS[33]**

  - **SODIUM AND WATER HOMEOSTASIS**

    - In most patients with stable CKD, the total-body content of Sodium and water is modestly increased.

- Normal renal function guarantees that the tubular reabsorption of filtered Sodium and water is adjusted so that urinary excretion matches net intake of Sodium and water.
- Many forms of renal disease (e.g.,Glomerulonephritis) disrupt this glomerulotubular balance such that dietary intake of Sodium exceeds its urinary excretion, leading to sodium retention and attendant extracellular fluid volume expansion.
- This expansion may contribute to hypertension, which itself can accelerate the nephron injury.
- As long as water intake does not exceed the capacity for water clearance, the extracellular fluid volume expansion will be isotonic and the patient will have a normal plasma Sodium concentration and effective osmolality.
- Hyponatremia is not commonly seen in CKD patients.

➢ POTASSIUM HOMEOSTASIS

- In CKD, the decline in GFR is not necessarily accompanied by a parallel decline in urinary Potassium excretion, which is predominantly mediated by Aldosterone-dependent secretory events in the distal nephron segments.
- Another defense against Potassium retention in these patients is augmented Potassium excretion in the gastro intestinal tract.

- ❖ Against defense mechanisms hyperkalemia may be precipitated by increased dietary Potassium intake, protein catabolism, hemolysis, hemorrhage, transfusion of stored red blood cells, and metabolic acidosis.
- ❖ Hypokalemia is not common in CKD and usually reflects markedly reduced dietary potassium intake, especially in association with excessive diuretic therapy or concurrent gastro intestinal losses.

➢ **METABOLIC ACIDOSIS**

- ❖ Metabolic acidosis is a common disturbance in advanced CKD.
- ❖ This is a non-anion-gap metabolic acidosis.
- ❖ With worsening renal function, the total urinary net daily acid excretion is usually limited to 30–40 mmol, and the anions of retained organic acids can then lead to an anion-gap metabolic acidosis.
- ❖ The non-anion-gap metabolic acidosis that can be seen in earlier stages of CKD may be complicated by the addition of an anion-gap metabolic acidosis as CKD progresses.
- ❖ In most patients, the metabolic acidosis is mild; the pH is rarely <7.35.

- **SKIN DISEASE**

Pruritus is common in severe CKD and is due to retention of nitrogenous waste products of protein catabolism. It improves following dialysis.

OTHER CAUSES OF PRURITUS IN CKD,

- Hypercalcemia
- Hyperphosphatemia
- Elevated calcium x phosphate product
- Hyperparathyroidism
- Iron deficiency

- **NEPHROGENIC SYSTEMIC FIBROSIS**

It is seen only in patients with moderate to severe CKD particularly in patients on dialysis. Skin is predominantly involved.

- **GASTROINTESTINAL COMPLICATIONS**
  - Decreased gastric emptying
  - Increased risk of reflux oesophagitis
  - Peptic ulceration
  - Acute pancreatitis
  - Constipation
  - Elevated serum amylase of upto three times normal, due to retention of high molecular weight forms of amylase in the body.

- **METABOLIC ABNORMALITIES**
  - Gout – Uric acid retention is a common feature of CKD.
  - Insulin- Insulin is catabolized by kidney and to some extent it is excreted by kidneys. End organ resistance to insulin is a feature of advanced CKD resulting in modestly impaired glucose tolerance.

- **ENDOCRINE ABNORMALITIES**

❖ Hyperprolactinaemia

❖ Increased Luteinizing hormone (LH) levels in both sexes and abnormal pulsality of LH release.

❖ Decreased serum Testosterone level, so erectile dysfunction and decreased spermatogenesis are common.

❖ Absence of normal cyclical changes in the female sex hormones resulting in oligo-menorrhoea or amenorrhoea.

❖ Abnormalities in Growth Hormone secretion and action resulting in impaired growth in uraemic children.

❖ Abnormal Thyroid hormone levels, partly because of altered protein binding.

- **MUSCLE DYSFUNCTION**

  Uremia interferes with muscle energy metabolism.

- **NERVOUS SYSTEM**

  Severe uremia causes an depressed cerebral function and decreased seizure threshold, asterixis , tremor and myoclonus.

- **NUTRITIONAL ABNORMALITIES**

➢ Protein-energy malnutrition, a consequence of low protein and caloric intake, is common in advanced CKD and is often an indication for initiation of Renal Replacement Therapy.

➢ These patients are resistant to the anabolic actions of insulin and other hormones and growth factors. Metabolic acidosis and the activation of inflammatory cytokines can promote protein catabolism.

- **ABNORMALITIES OF LIPID METABOLISM[34]**

Progressive deterioration of renal function results in altered composition of blood lipids which in turn predisposes to the development of cardio vascular disease. Renal dyslipidemia is characterized by the following features:

❖ Hepatic apo AI synthesis is decreased and Lecithin cholesterol acyl transferase activity is reduced. This leads to decreased HDL-C levels.

❖ Increased synthesis of apo C III, a competitive inhibitor of Lipoprotein Lipase leads to elevated levels of VLDL-C and Chylomicrons, which results in hypertriglyceridemia. Further, uremic toxins and secondary hyperparathyroidism reduces the levels of lipoprotein lipase which results in impaired catabolism of Triglyceride rich lipoproteins.

- ❖ Total and LDL- Cholesterol levels are usually normal but may be low in patients with concomitant inflammation and malnutrition. There is characteristic accumulation of small dense atherogenic LDL-C[35].
- ❖ As GFR declines, the levels of high molecular weight isoforms of Lipoprotein (a) increase which is associated the increased cardiovascular risk.
- ❖ The changes in lipoprotein composition and structure in CKD stimulate and amplify the already existing inflammatory mechanisms which in turn results in endothelial dysfunction and atherosclerotic progression.

- **CARDIO VASCULAR DISEASE[36]**

Cardiovascular disease is the most frequent cause of death of patients with Chronic Kidney Disease. The incidence of CVD is seven to tenfold greater in patients with CKD than in non-CKD patients.

Potential mechanisms for increased cardiovascular disease risk in chronic kidney disease[37]

1. Chronic kidney disease is associated with an increase in prevalence of cardiovascular disease risk factors and these CVD risk factors promote development and progression of CKD.
2. Cardiovascular disease is a risk factor for CKD.
3. Chronic kidney disease is an independent risk factor for cardiovascular disease.

Cardiovascular disease is characterized by left ventricular hypertrophy which results largely due to expansion of extracellular volume, anemia and hypertension. Left ventricular remodelling and fibrosis may accompany left ventricular hypertrophy which ultimately results in severe complications[38,39].

Cardiovascular complications associated with CKD include Myocardial infarction, Angina pectoris, Arrhythmias, Cardiac failure, Peripheral Vascular Disease, Stroke and Sudden death. The risk increases from early stages to advanced stages of CKD[40].

CARDIOVASCULAR RISK FACTORS IN CKD[41,42,43]

TRADITIONAL RISK FACTORS

- Age
- Gender
- Diabetes Mellitus
- Hypertension
- Smoking

NON-TRADITIONAL RISK FACTORS

- Inflammation
- Oxidative stress
- Endothelial dysfunction
- Anaemia

- Hyperphosphatemia
- Secondary hyperparathyroidism
- Vascular calcification
- Advanced glycation end products
- Hyper homocystinemia

- **CHRONIC INFLAMMATION IN CKD[44]**

Most CKD patients are in a state of chronic inflammation. Recurrent or chronic inflammatory processes are common in individuals in both non dialysis dependent CKD and ESRD undergoing dialysis. Chronic inflammation is a major contributor to increased mortality and morbidity in end stage renal disease.

- **POTENTIAL CONTRIBUTORS OF INFLAMMATION IN CKD**

1. Decreased clearance of pro inflammatory cytokines;

   Serum CRP, IL-6 and hyaluronan levels are inversely correlated with creatinine clearance.

2. Volume overload;

   Vascular congestion from fluid overload may result in altered permeability of the gastrointestinal tract, thereby leading to accumulation of endotoxins such as lipopolysaccharides and bacteria. This process may in turn stimulate monocytes and the increased release of proinflammatory cytokines.

3. Oxidative and carbonyl stress;

   Increased production of cytokines induced by oxidative stress is observed in patients with CKD and ESRD. Oxidative stress is an important condition for the development of endothelial dysfunction, inflammation and atherogenesis. Low plasmologens is an indicator of such stress reported in malnourished patients with CKD. Advanced glycosylated end products(AGE) resulting from carbonyl stress initiate inflammation in patients with CKD and especially in patients with ESRD. It may be due to

   - Decreased antioxidant levels
   - Co-morbid conditions
   - Deteriorating protein-energy nutritional state and food intake
   - Genetic factors such as low production of anti-inflammatory cytokines
   - Other factors like autoimmune diseases (etiology of CKD), genetic factors, unrecognized persistent infections and atherosclerosis may also underlie inflammation among patients with CKD or ESRD.

BETA-TRACE PROTEIN

DISCOVERY

Beta-trace protein (BTP), first described in 1961 by J. Clausen, is a monomeric glycoprotein that belongs to the lipocalin superfamily[45,46].

In 1993, an amino acid sequence determination revealed that BTP has a prostaglandin synthase activity (prostaglandin-H2 D-isomerase) and catalyses the conversion of prostaglandin H2 (PGH2), a common precursor of various prostanoids, to prostaglandin D_2 (PGD2)[45,47,48,49,50]. It is otherwise known as Lipocalin-type prostaglandin D synthase (L-PGDS)[51,52].

PGD2 is involved in sleep induction and regulation, adipocyte differentiation, nociception, bronchoconstriction, inflammatory mediator modulation, nitric oxide release, induction of vasodilation, and inhibition of platelet aggregation[45,46].

STRUCTURE

It is a monomeric glycoprotein with 168 amino acids and an estimated molecular mass between 23,000 and 29,000 Da, depending whether it is N-glycosylated at two positions, Asn51 and Asn78[47].

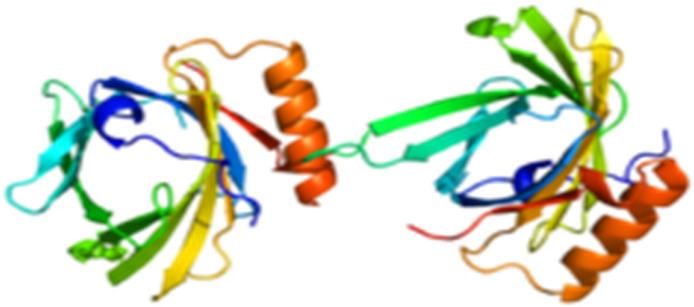
CSF-derived BTP is a glycoprotein exhibiting significant micro heterogeneity which is due to special truncated biantennary oligosaccharide chains that have four following characteristics:[53]

i. high amounts of terminal galactose (Gal) residues
ii. high amounts of terminal Nacetylglucosamine (GlcNAc) (agalactoantennae as well as bisecting GlcNAc)
iii. proximal α l,6-fucosylation
iv. notable amounts of Lewis-type peripheral fucosylation (fucose bound α 1,3 to GlcNAc)

Neuraminic acid (NeuAc) is present in α 2,3and α 2,6-linkage in approximately equal amounts.

FIGURE 3

CRYSTAL STRUCTURE OF BTP



SECRETION AND EXPRESSION

Two major forms are recognized[11,54,55]

- Brain-type BTP is a member of the lipocalin superfamily, and
- Hematopoietic BTP is a member of the glutathione synthase class.

BTP brain-specific is one of the major polypeptide constituents of the cerebrospinal fluid (CSF), and is found in much lower concentrations in the blood.

Serum BTP assays measure only brain-type BTP, which is produced by the epithelial cells of the choroid plexus in the central nervous system. From the cerebrospinal fluid, it diffuses into the systemic circulation. After diffusion, the liver rapidly eliminates the non-sialylated "brain type" glycoforms by specialized receptors, resulting in larger "blood/urine" sialylated glycoforms[46]. It binds to lipophilic substances in blood, such as retinoids, thyroid hormone, bilirubin, biliverdin, gangliosides and amyloid-beta peptides[56].

BTP has been found to be expressed in the brain, retina, melanocytes, male genital organs, heart, and kidneys and is secreted into various body fluids, such as cerebrospinal fluid, seminal fluid, plasma and urine[57,58].

In the brain, BTP is secreted mainly by the leptomeninges and to a certain degree by the choroid plexus[59,60].

BIOLOGICAL FUNCTIONS OF BTP:

MARKER OF CSF OTORROHEA AND RHINORRHOEA

As BTP produced by leptomeninges and choroid plexus, it is used as a marker of CSF leakage[60].

ROLE OF BTP IN HEART

In human heart, BTP is located in myocardial cells, atrial and ventricular endocardial cells, both coronary arteries, smooth muscle cells and atherosclerotic plaque. Therefore BTP levels are elevated in circulation of patients with severe coronary heart disease[57].

BTP exerts significant effects on the cardiovascular system, including 15-deoxy-D12,14-PGJ_2 (15d-PGJ_2), the most effective endogenous activator for the nuclear receptor peroxisome proliferator-activate receptor γ (PPARγ). 15d-PGJ_2 promotes several processes in vascular cells, such as antiatherogenic, antithrombogenic, antiapoptotic, and antiinflammatory effects. In the same way, PGD_2 may suppress inflammatory processes and prevent platelet aggregation, and it decreases the marker of fibrinolytic system plasminogen activator inhibitor-1 (PAI-1) mRNA expression and PAI-1 synthesis in cultured bovine endothelial cells after incubation with IL-1β[61].

ROLE OF BTP IN HEART IN THE CONTEXT OF HYPOXIA AND ISCHEMIA[61]

Pulmonary hypertension caused by chronic hypoxemia can eventually lead to heart failure because of pressure overload of the right ventricle. In chronic hypoxemia, expression of BTP mRNA and protein level in the heart is correlated with hypoxic conditions, and BTP values tends to increase under hypoxia. This increased expression in the heart may reflect an adaptive mechanism to pressure overload. The cardioprotective effects of PGD_2 are likely mediated through the D-type prostanoid receptor, a G protein-coupled receptor. After PGD_2-D-type prostanoid receptor binding, extracellular signal-regulated kinase ½ activation leads to cardiomyocyte protection.

ROLE OF BTP IN ISCHEMIC HEART DISEASE[61]

BTP has intimate roles in the pathophysiology of vascular disease and atherosclerosis. In patients with stable angina, plasma level of BTP is higher in the cardiac vein than coronary arteries. Hence, BTP concentrations in the cardiac vein decreases after percutaneous coronary intervention. Similarly, it has also been observed that an increase in serum BTP levels 48 hours after percutaneous coronary intervention correlates with restenosis rates.

Regarding vasomotor reactivity, coronary spasm plays an important role in the pathogenesis of ischemic heart disease. Currently, there are no established biomarkers that assess the presence of vasospastic angina (VSA), and endothelial dysfunction has been implicated as a crucial factor in the pathogenesis of coronary vasospasm in patients with VSA. Shear stress stimulates PGD_2 synthesis by BTP expression in vascular endothelial cells in response to blood flow, and vasoconstriction can reduce blood flow, thus increasing arterial shear stress. It has been observed that serum levels of BTP are elevated in patients with VSA, and they are associated with the degree of coronary vasoconstriction in response to acetylcholine, a pharmacological tool used to induce coronary vasospasms and evaluate endothelial function.

ROLE OF BTP IN HYPERTENSION[62]

Serum and urinary BTP values are much higher in patients with essential hypertension, even in normal kidney function. Moreover, hypertension with renal injury is associated with further increased BTP concentrations in serum and urine.

This increased serum BTP is associated with urinary excretion of BTP, and consistent with its role as a sensitive kidney function marker, urinary BTP precedes an increase in urinary albumin excretion. This increase probably reflects injuries in the renal tubules and arterioles induced by hypertension.

ROLE OF BTP IN KIDNEY

BTP is a low molecular weight protein, freely filtered by the glomerulus without secretion and/or reabsorption in renal tubules and it is almost completely excreted via the kidneys. Increased concentrations of BTP in serum reflect reduced clearance of the protein. The half life of BTP is approximately 1.2 hours and there is minimal extrarenal clearance[10].

Albuminuria is a well known biomarker of kidney damage that occurs when glomerular function is normal, but the proximal tubules have a diminished capacity to reabsorb and catabolize proteins, causing an increased urinary excretion of proteins that usually pass through the glomerulus[63].

Serum BTP concentration is not correlated with C-reactive protein and inflammation[64].

This marker is not changed by body composition changes[65].

Its serum concentration is unaffected by thyroid function and corticosteroid administration[61].

It is a better GFR marker in newborns than creatinine and cystatin C[66].

Because of its lower molecular mass, its anionic property, its constant production rate and its stability[63], assay of serum BTP is utilized to detect renal disease in the present study.

AIMS AND OBJECTIVES

AIM:

To estimate the levels of Serum Beta-Trace Protein in patients with CKD and to compare them with healthy normal subjects

OBJECTIVES:

1. To estimate the serum level of Beta-Trace Protein in patients with chronic kidney disease
2. To correlate the serum Beta-Trace Protein level with serum creatinine and Creatinine clearance
3. To evaluate the correlation between serum Beta-Trace Protein level and other several known risk factors such as Body mass Index, Blood pressure, Random blood sugar and blood urea

MATERIALS AND METHODS

The study was conducted at Thanjavur Medical College Hospital, Thanjavur after getting approval from the ethical committee.

50 patients of known CKD (25 males and 25 females) were selected as cases from the outpatients and wards of the Department of Nephrology. 50 age and gender matched healthy individuals were selected as controls.

INCLUSION CRITERIA

- Patients with established diagnosis of CKD.
- Age > 18 years

EXCLUSION CRITERIA

1. Primary tubular diseases
2. Recent or concurrent administration of potentially nephrotoxic drugs
3. Acute kidney injury
4. Terminal kidney failure requiring dialysis
5. Patients with known neurological disease

SAMPLE COLLECTION:

Informed consent was obtained from all subjects prior to the study. Under aseptic precautions, 5ml of venous blood sample was collected after an overnight fasting of 12 hours from all subjects. After retraction of the clot, samples were centrifuged at 2000rpm for 15 minutes for separation of serum.

An aliquot of the serum was taken for the estimation of Beta-Trace Protein and stored at -20°C in the deep freezer. The remaining serum was used for the estimation of Glucose, Urea and Creatinine.

ANALYSIS OF BLOOD SAMPLES

The serum collected above was used for the estimation of the following parameters.

A. ESTIMATED PARAMETERS

1. Serum BTP	Enzyme Immuno Assay
2. Blood Urea	Urease –Glutamate Dehydrogenase (GLDH) Method
3. S. Creatinine	Modified Jaffe's method.
4. Glucose	Glucose-oxidase/ peroxidase method

B. CALCULATED PARAMETERS

1. Creatinine clearance was calculated using Cockcroft- Gault formula

$$\text{Estimated Creatinine Clearance} = \frac{(140\text{-age}) \text{ X wt in kg}}{72 \text{ X serum Creatinine}}$$

Multiply by 0.85 for females.

ESTIMATION OF SERUM BETA-TRACE PROTEIN

Method:

Enzyme Immuno Assay Kit Purchased from Aviva systems biology

Principle:

An antibody specific for PTGDS has been pre-coated onto a well plate. Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for PTGDS is added, incubated and followed by washing. Avidin Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample PTGDS captured in the well.

REAGENTS:

1. PTGDS Microtiter plate: 96 wells coated with secondary antibody.
2. Lyophilized standard PTGDS - 2 vials
3. 100X Biotinylated PTGDS Detector antibody – 1x120 µL
4. 100X Avidin-HRP Conjugate – 1x120 µL

5. Sample Diluent – 1x20 mL

6. Detector Antibody Diluent – 1x10 mL

7. Conjugate Diluent – 1x10 mL

8. Wash buffer concentrate 25X - 30ml

9. Tetra methyl Benzidine (TMB) Substrate – 1x10 mL

10. Stop solution – 1x10 mL

Reagent Preparation:

1. All materials were equilibrated to room temperature prior to use

2. Preparation of 1X Biotinylated PTGDS Detector Antibody:

1X Biotinylated PTGDS Detector Antibody was prepared by diluting the 100X Biotinylated PTGDS Detector Antibody 1:100 with detector antibody diluent

3. Preparation of 1X HRP-Avidin Conjugate:

1X HRP-Avidin Conjugate was prepared by diluting 100X Avidin-HRP conjugate 1:100 with conjugate diluent

4. Preparation of Wash Buffer:

30 ml of wash buffer was added to 720 ml of ultra-pure water

5. Preparation of Standards:

Reconstituted one vial of 50 ng Lyophilized PTGDS standard by adding one ml of sample diluent

8 micro-tubes were labeled with the following concentrations:

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	50,000 pg/mL Reconstituted PTGDS Standard	1,000	NA	NA	50,000 pg/mL
2	50,000 pg/mL	300	300	600	25,000 pg/mL
3	25,000 pg/mL	300	300	600	12,500 pg/mL
4	12,500 pg/mL	300	300	600	6,250 pg/mL
5	6,250 pg/mL	300	300	600	3,125 pg/mL
6	3,125 pg/mL	300	300	600	1,560 pg/mL
7	1,560 pg/mL	300	300	600	780 pg/mL
8	NA	0	300	300	0.0 (Blank)

6. TMB Substrate : ready to use

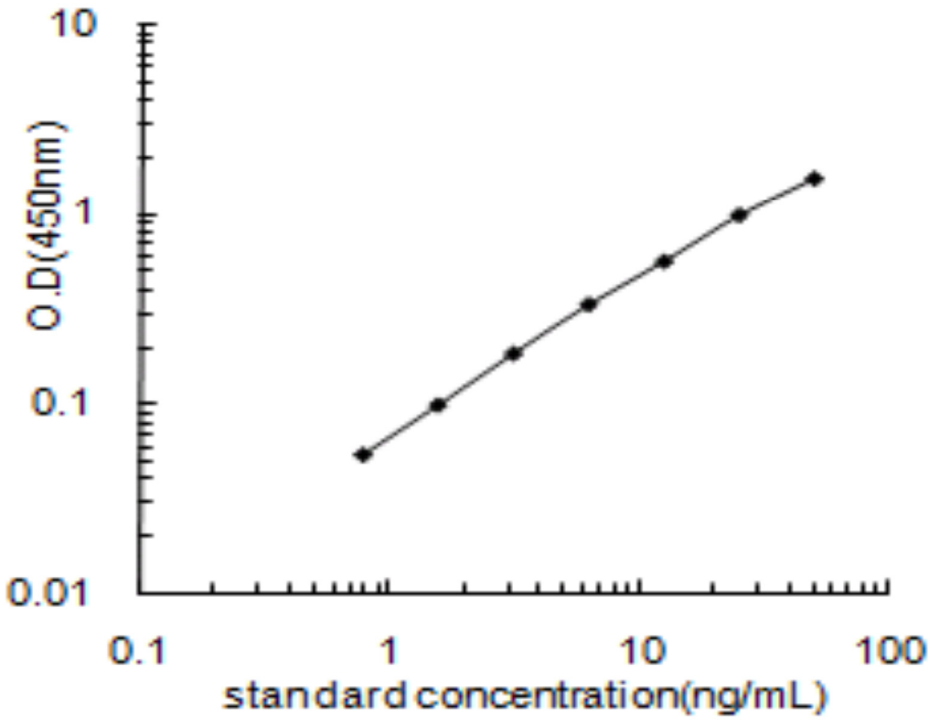
7. Stop solution : ready to use

Assay Procedure:

1. Micro-titre plate was equilibrated to room temperature before opening the sealed pouch.
2. 100µl of serially titrated standards and samples were added into wells. Then the plate was incubated for 2 hours at room temperature with gentle shaking (1-2 cycles/sec)
3. After incubation the solution was discarded and the plate was inverted and blotted against clean paper towels
4. 100 µl of prepared 1X Biotinylated PTGDS Detector Antibody was added to each well and incubated at room temperature for 60 minutes
5. After Incubation the solution was discarded and washed 3 times with wash buffer
6. 100µl of prepared 1X Avidin-HRP conjugate solution was added to each well and incubated for 60 min with gentle shaking at room temperature
7. After Incubation the solution was discarded and washed 5 times with wash buffer
8. 90µl of TMB substrate was added to each well and incubated for 30 min at room temperature in the dark with gentle shaking (1-2 cycles/sec.)
9. 50µl of stop solution was added and absorbance was read at 450nm immediately

Calibration Graph:

By plotting the mean absorbance of each standard on the y-axis against the concentration of BTP (ng/ml) in each standard on the x-axis, a calibration curve was constructed.



Sensitivity:

The minimum detectable concentration of BTP is **< 0.25 ng/ml**

Assay Range: 0.78 - 50 ng/ml

ESTIMATION OF UREA

Method:

Urease – GLDH method

Principle:

Urea is hydrolyzed to ammonia and carbon dioxide by Urease. Then Glutamate dehydrogenase (GLDH) converts Ammonia and α Keto glutarate to Glutamate and water with the concurrent oxidation of NADH to NAD^+ . Two moles of NADH are oxidized for each mole of urea present.

$$\text{Urea} + H_2O \xrightarrow{Urease} 2NH_4^+ + CO_2 .$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow{GLDH} \text{Glutamate} + NAD^+.$$

The initial rate of decrease in absorbance at 340nm is proportional to the Urea concentration in the sample.

Reagent composition:

Reagent 1:

α-Keto Glutaric Acid 99.8mmol/L

Urease 23.5kU/L, GLDH 3.5KU/L, Adenosine diphosphate 7.6mmol/L, Sodium Azide 0.2%.

Reagent 2:

NADH 2.95mmol/L, sodium Azide 0.1%

Concentration of Urea standard 50mg/dl.

Reagent Preparation:

Working reagent was prepared by mixing 4 parts of reagent 1 with one part of reagent 2.

Procedure:

3 test tube were labeled as Blank, standard and test and the procedure is done as follows:

Tubes	**Working reagent**	**Standard**	**Test sample**	**Distilled water**
Blank	1000µl	-	-	10µl
Standard	1000µl	10µl	-	-
Test	1000µl	-	10µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 60 sec (A_2) at 340nm.

Calculation:

$\Delta A = A_2 - A_1$.

$$\text{Urea in mg/dl} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of standard}$$

Linearity:

The method is linear upto 200mg/dl.

Reference Interval:

Adults 13 - 45mg/dl.

ESTIMATION OF SERUM CREATININE

Method:

Modified Jaffe's Method.

Principle:

Creatinine reacts with alkaline picrate to produce an orange-yellow colour. The intensity of the colour is directly proportional to the concentration of Creatinine and is measured photometrically at 510nm.

Reagent Composition

Reagent No	Composition	Concentration
1	Picric acid	25.8mmol/L
2	Sodium hydroxide	95mmol/L

Concentration of standard creatinine 2 mg/dl

Reagent Preparation

Equal Volumes of reagent 1 and reagent 2 were mixed and waited for 15 minutes before use.

Procedure

3 test tubes were taken and labeled as Blank, Standard and test and the procedure was done as follows:

Tubes	Working reagent	Standard	Test sample	Distilled water
Blank	1000µl	-	-	100µl
Standard	1000µl	100µl	-	-
Test	1000µl	-	100µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 80 sec (A_2) at 510nm, against reagent blank with distilled water.

Calculation

$\Delta A = A_2 - A_1$.

$$\text{Serum Creatinine (mg/dl)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of standard}$$

Linearity

This assay is linear upto 20mg/dl.

Reference Range

Males: 0.7-1.4 mg/dl

Females: 0.6-1.2mg/dl.

ESTIMATION OF GLUCOSE

Method:

Glucose oxidase – peroxidase (GOD-POD) method.

Analysis:

End Point Analysis

Principle:

Glucose is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide Oxidatively couples with 4-amino Antipyrine and phenol to produce red quinoneimine dye in the presence of peroxidase. This red dye has maximum absorbance at 505nm. The intensity of the colour complex is directly proportional to the concentration of glucose in specimen.

$$\text{D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4- Amino Antipyrine} + \text{Phenol} \xrightarrow{POD} \text{red dye} + H_2O$$

Specimen:

Fresh unhemolysed serum

Assay Procedure:

Enzyme reagent and standard were brought to the room temperature before performing the assay.

Reagents	**Blank**	**Standard**	**Sample**
Glucose enzyme reagent	1000µl	1000µl	1000µl
Standard	-	10µl	-
Sample	-	-	10µl
Distilled water	10µl	-	-

The tubes were mixed thoroughly and incubated at 37^{o} for 10min.

The absorbance was read against reagent blank at 505nm.

Calculation:

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (mg/dl)}$$

Glucose Standard: 100mg/dl

Linearity: Upto 500mg/dl

Normal Values:

Glucose fasting: 65-100mg/dl

Glucose postprandial: 90-140mg/dl

RESULTS

A total of 100 subjects were selected as the study group for the present study. This includes 50 cases with Chronic Kidney Disease and 50 age matched healthy controls.

Levels of serum Beta-Trace Protein, Urea, Creatinine, and Random Blood Sugar were estimated for all the samples of the study group. Creatinine clearance were calculated from the formula.

The values obtained in controls and cases are presented in the master chart I and II respectively.

STATISTICAL ANALYSIS

- Student's t-test was employed for the statistical analysis of data.
- The data were expressed in terms of mean and standard deviation.
- 'P' value less than 0.05 was taken as the significant value.
- Correlation between the measured parameters was assessed using Pearson's correlation coefficient.

Table 1:

Represents the minimum level, maximum level, mean and SD of all parameters of the study group.

Table 2:

Shows comparison of serum BTP in cases and controls

The mean value of serum BTP in cases was 52.24 ng/ml and this was significantly higher than that of control group (33.86 ng/ml; 'p' < 0.001)

Table 3 :

Shows comparison of serum creatinine in cases and controls

There was increase in the mean serum creatinine in cases (1.41 ± 0.84), when compared to controls (0.92 ± 0.15), which is statistically significant (p value < 0.001)

Table 4 :

Shows comparison of Blood urea in cases and controls

There was increase in the mean blood urea in cases (60.7 ± 40.15), when compared to controls (37.5± 4.82), which is statistically significant. (p value < 0.001)

Table 5:

Shows comparison of Creatinine clearance among cases and controls

Ccr was significantly lower in cases (43.32 ± 12.8) than controls (115.13 ± 8.6)

Table 6:

Shows comparison of RBS in cases and controls

There was increase in the mean RBS in cases (110.7 ± 35.14), when compared to the mean RBS in controls (93.94± 6.97), which is statistically significant. (p value < 0.001)

Table 7 :

Shows comparison of Systolic BP in cases and controls

There was increase in the mean SBP in cases (117.62 ± 8), when compared to controls (130.04 ± 26.03), which is statistically significant. (p value < 0.001)

Table 8 :

Shows comparison of Diastolic BP in cases and controls

There was increase in the mean DBP in cases (83.56 ± 11.1), when compared to controls (78.08 ± 4.4), which is statistically significant.

(p value < 0.001)

Table 9:

Shows comparison of BMI in cases and controls

There was increase in the mean BMI in cases (25.2 ± 2.87), when compared to the mean BMI in controls (24.82 ± 1.73), which is not statistically significant. (p value >0.05)

Table 10:

Shows the age distribution among cases and controls

The mean age falls between 51.88 ± 4.50.

Table 11:

Gender wise distribution of the study group

The distribution of males and females in the study group were 50% and 50% respectively which shows equal distribution

Table 12:

Comparison of serum BTP in the study group in relation to creatinine clearance

There was a progressive increase in serum Beta-Trace Protein levels as renal function declined.

Table 13:

Karl Pearson Coefficient correlation between serum BTP and other Biochemical parameters in cases

There was significant positive correlation between S.BTP with S.creatinine, Blood urea, RBS and negative correlation with Creatinine clearance

Figure 4:

Bar diagram showing comparison of serum BTP values among cases and controls

Figure 5:

Bar diagram showing comparison of serum creatinine in cases and controls

Figure 6:

Bar diagram showing comparison of Blood urea in cases and controls

Figure 7:

Bar diagram showing comparison of Ccr in cases and controls

Figure 8:

Bar diagram showing comparison of RBS in cases and controls

Figure 9:

Bar diagram showing comparison of Systolic BP in cases and controls

Figure 10:

Bar diagram showing comparison of Diastolic BP in cases and controls

Figure 11:

Bar diagram showing comparison of BMI in cases and controls

Figure 12:

Bar diagram showing age distribution among cases and controls

Figure 13:

Bar diagram showing comparison of serum BTP in the study group in relation to Ccr.

Table 1

Descriptive Statistics of controls and cases

Variables	Control (n=50)				Cases (n=50)			
	Min	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**
AGE	43	61	51.88	4.5	43	61	51.18	4.08
WT	50	80	66.6	7.19	55	86	71.26	7.3
HT	1.45	1.72	1.61	0.08	1.5	1.87	1.67	0.07
BMI	21	29	24.82	1.73	17	31	25.2	2.87
SBP	100	130	118	8	90	180	130	26.03
DBP	70	84	78	4.46	70	110	84	11.14
BTP	10	50	33.86	12.1	12	149	52.4	29.6
CREATININE	0.6	1.2	0.92	0.15	0.6	5.2	1.41	0.84
Ccr	95.08	133.7	115.13	8.6	4.55	66.56	43.32	12.8
UREA	23	44	37.5	4.82	28	179	60.7	40.15
RBS	80	106	93.94	6.97	76	220	110.7	35.14

TABLE 2

Comparison of S. BTP among cases and controls

T-TEST			
S. BTP (ng/ml)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	33.86	12.1	p value < 0.001 Significant
Cases (n=50)	52.24	29.6	

FIGURE 4

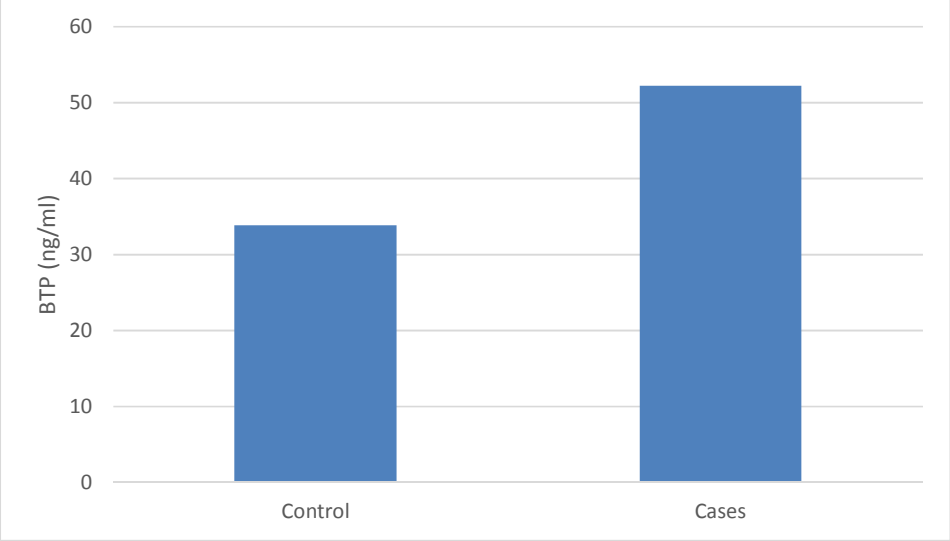


TABLE 3

Comparison of S. Creatinine among cases and controls

T-TEST			
S.Creatinine (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	0.89	0.13	p value < 0.001 Significant
Cases (n=50)	4.7	2.5	

FIGURE 5

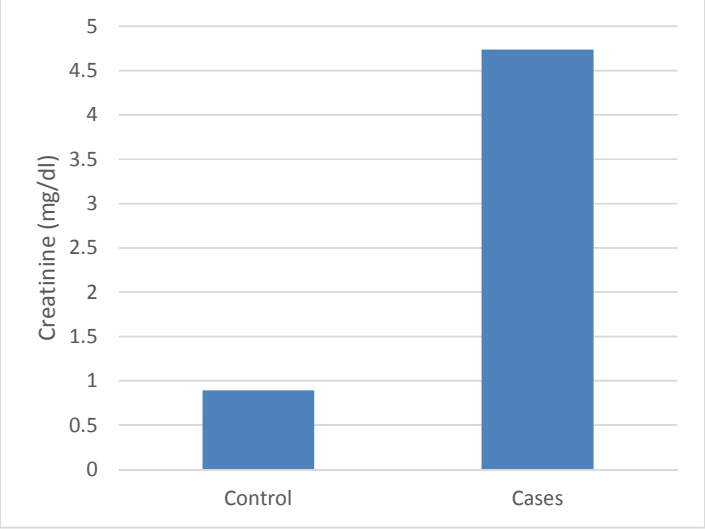


TABLE 4

Comparison of Blood urea among cases and controls

T-TEST			
Urea (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	37.5	4.82	p value < 0.001 Significant
Cases (n=50)	60.7	40.15	

FIGURE 6

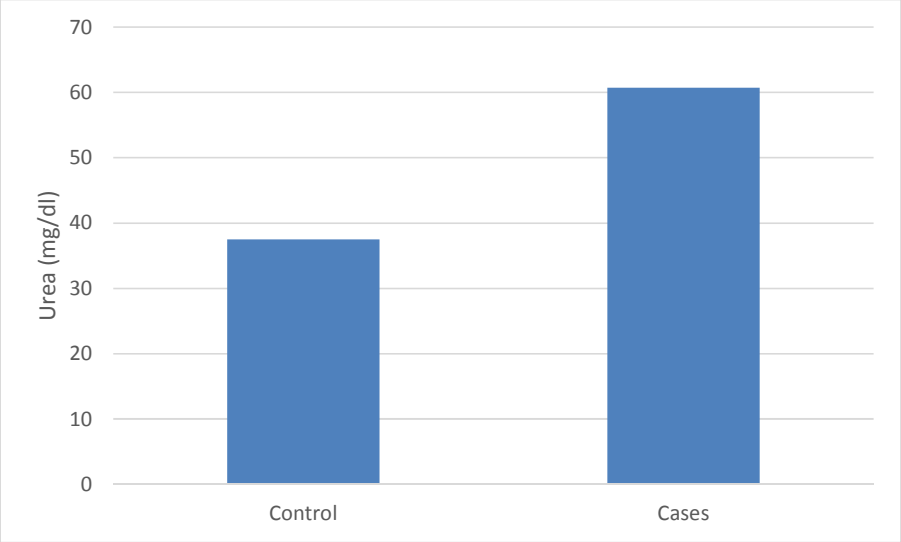


TABLE 5

Comparison of Creatinine clearance among cases and controls

T-TEST			
Ccr (ml/min)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	115.13	8.6	p value < 0.001 Significant
Cases (n=50)	43.32	12.8	

FIGURE 7

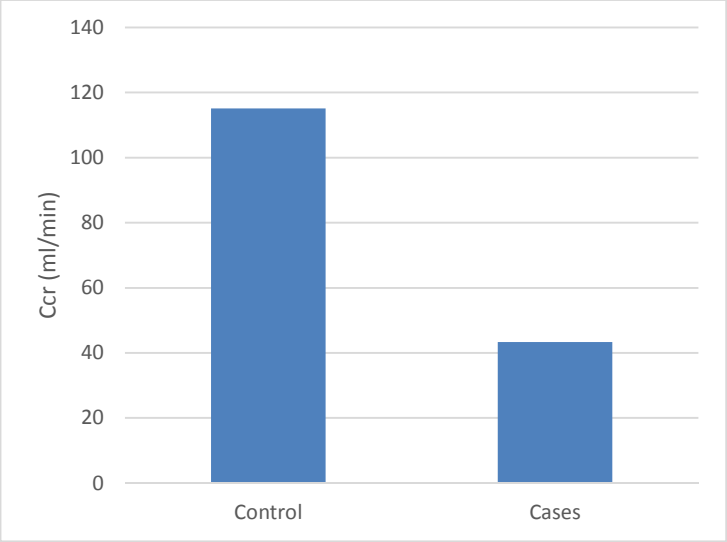


TABLE 6

Comparison of RBS among cases and controls

T-TEST			
RBS (mg/dl)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	93.94	6.97	p value < 0.001 Significant
Cases (n=50)	116.96	39.8	

FIGURE 8

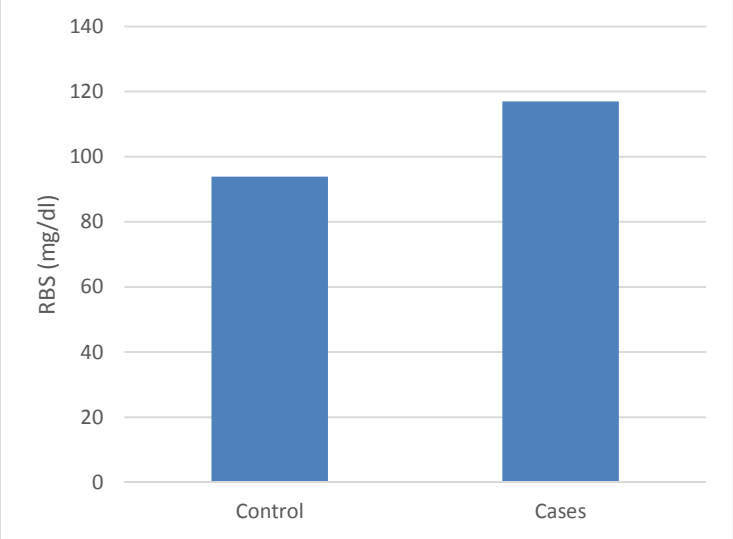


TABLE 7

Comparison of SBP among cases and controls

T-TEST			
SBP (mm/Hg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	118	8	p value < 0.001 Significant
Cases (n=50)	130	26.03	

FIGURE 9

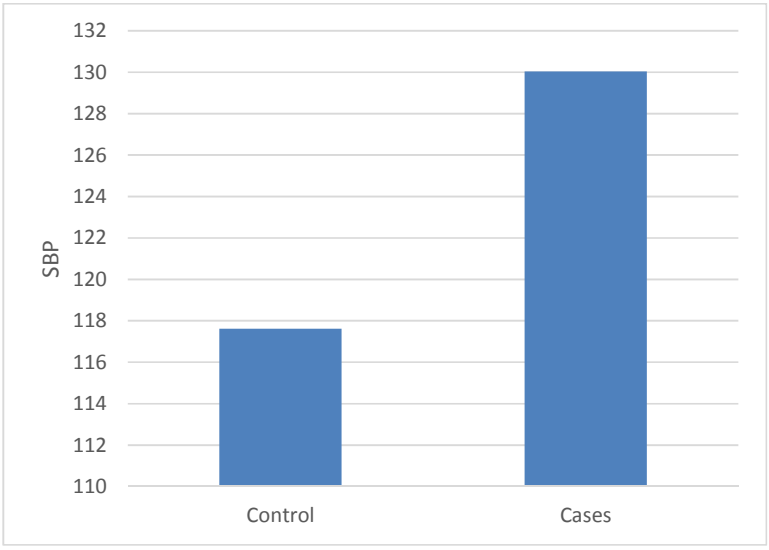


TABLE 8

Comparison of DBP among cases and controls

T-TEST			
DBP (mm/Hg)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	78	4.46	p value < 0.001 Significant
Cases (n=50)	84	11.14	

FIGURE 10

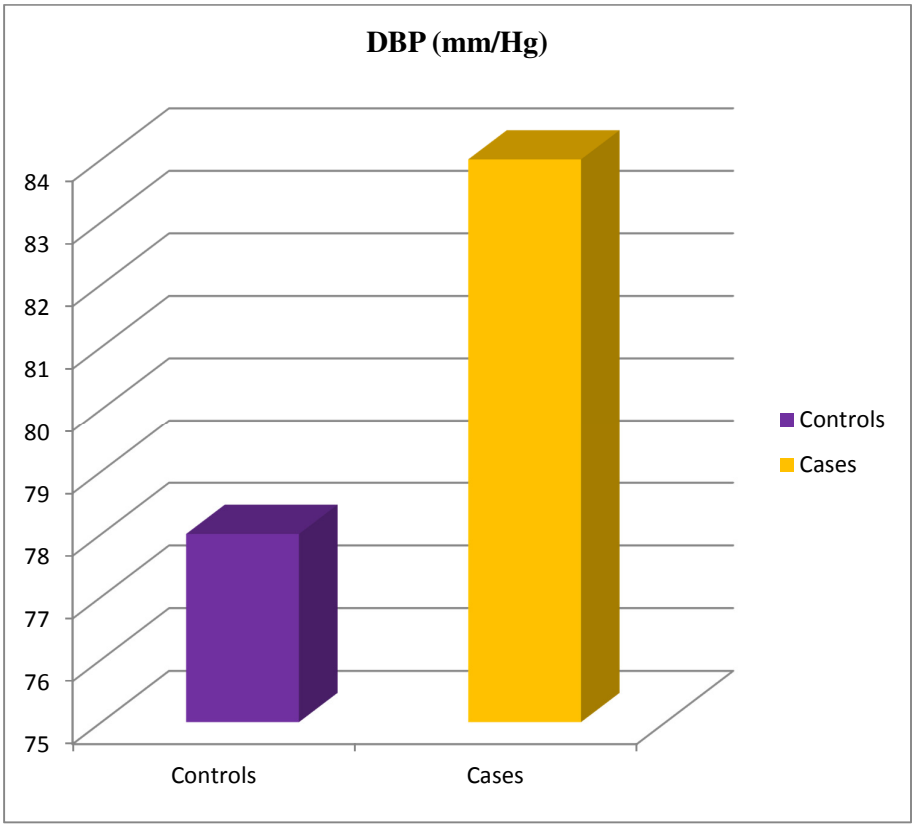


TABLE 9

Comparison of BMI among cases and controls

T-TEST			
BMI (Kg/m^2)	**MEAN**	**SD**	**STATISTICAL INFERENCE**
Control (n=50)	24.82	1.73	p value >0.05 Non Significant
Cases (n=50)	25.2	2.87	

FIGURE 11

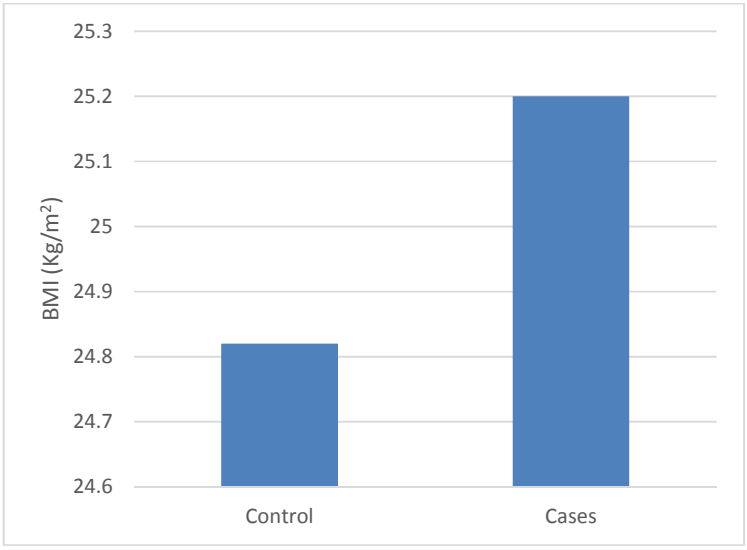


TABLE 10

Age Distribution among control and cases

AGE	CONTROL(n=50)		CASES(n=50)		TOTAL(n=100)	
41-50 yrs	17	34%	19	38%	36	36%
51-60 yrs	31	62%	30	60%	61	61%
> 60 yrs	2	2%	1	2%	3	3%

FIGURE 12

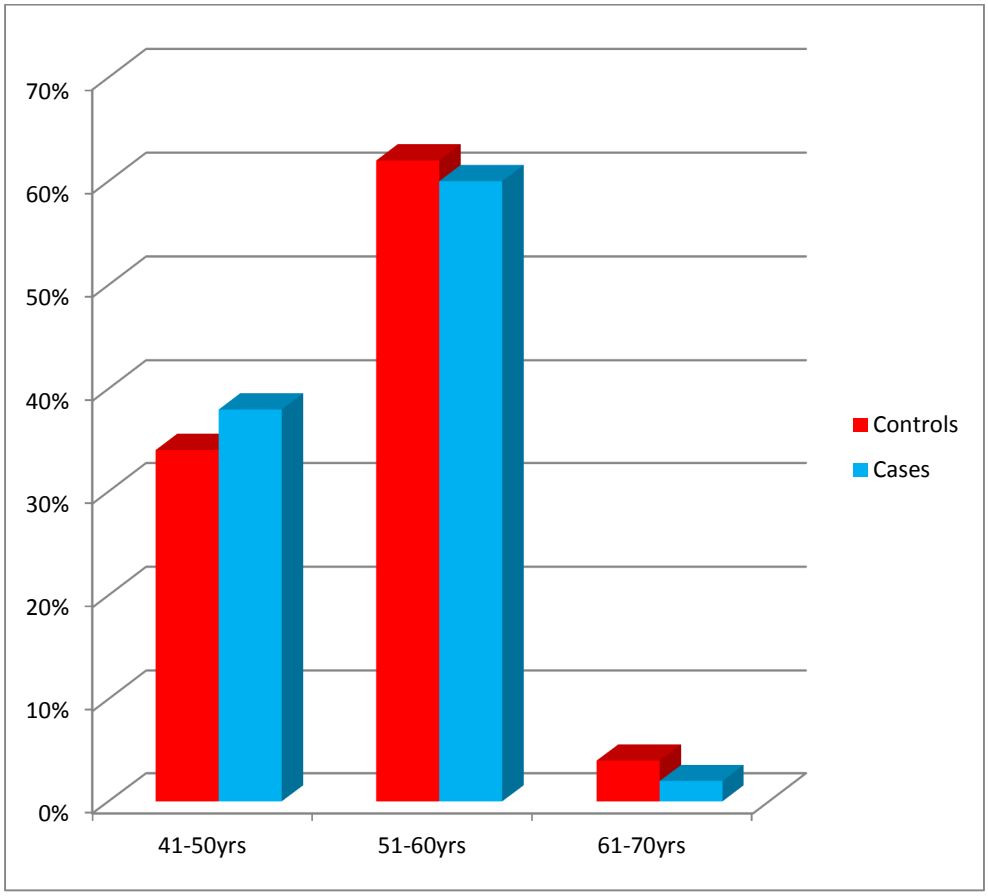


TABLE 11:

Gender wise distribution of the study group

GENDER			
STUDY GROUP	**MALES (%)**	**FEMALES(%)**	**STATISTICAL INFERENCE**
Control (n=50)	25 (50%)	25 (50%)	p value >0.05 Non Significant
Cases (n=50)	25 (50%)	25 (50%)	

TABLE 12

Comparison of serum BTP in the study group in relation to creatinine clearance

Creatinine Clearance (ml/min)	**BTP (ng/ml)**	
	Mean	**S.D.**
60-90 (n=5)	26.6	5.2
30-59 (n=37)	53.51	20.1
15-29 (n=6)	72.1	8.1
<15 (n=2)	147	2.8

FIGURE 13

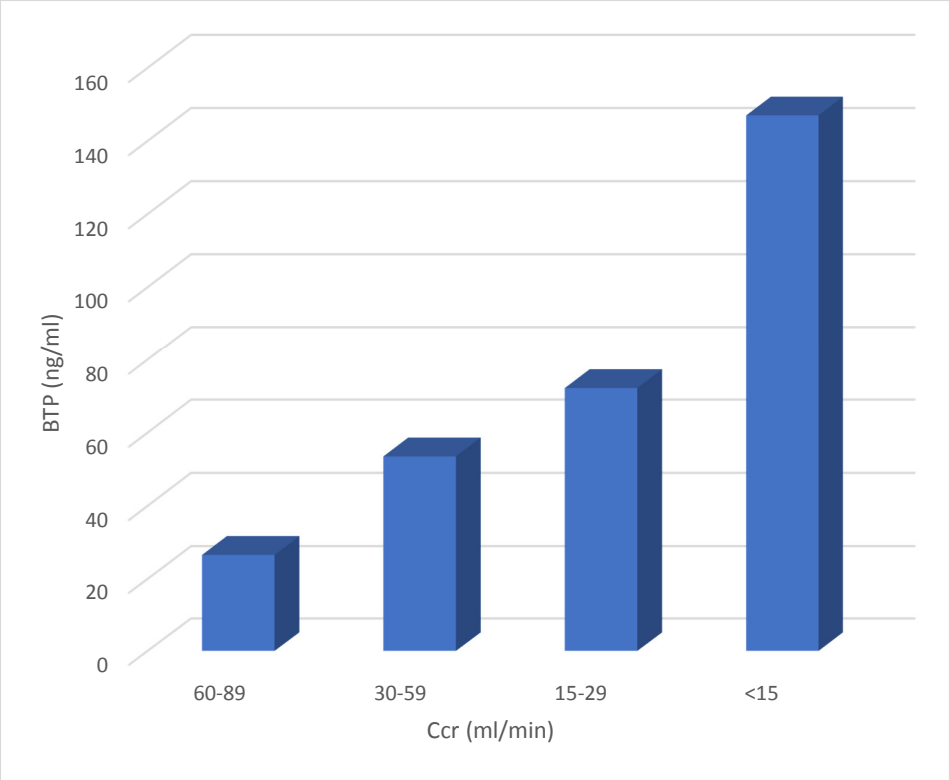

160
140
120
100
80
60
40
20
0
BTP (ng/ml)
60-89
30-59
15-29
<15
Ccr (ml/min)

TABLE 13

Karl Pearson Coefficient correlation between serum BTP and other parameters in cases

PARAMETERS	**CORRELATION COEFFICIENT (R)**	**CORRELATION**
CREATININE	0.630	Correlated
Ccr	-0.765	Correlated
UREA	0.721	Correlated
RBS	0.700	Correlated
SBP	0.009	Not Correlated
DBP	0.046	Not Correlated

DISCUSSION

Chronic kidney disease is a clinical syndrome that occurs when there is a gradual decline in renal function over time. Early detection and treatment are needed to prevent progression to kidney failure and complications such as coronary vascular disease.

Till date standard test to detect Chronic Kidney Disease is serum creatinine. However measurement of creatinine is a crude marker, detecting changes in renal function only when there is 50% reduction in GFR.

In the present study serum Beta-Trace Protein concentrations were found to be significantly increased in patients with Chronic Kidney Disease (mean 52.24 ± 29.6) when compared to the control group (mean 33.86 ± 12.1).

When patients in different stages of Chronic Kidney Disease were compared, serum Beta-Trace Protein levels were found to be progressively increased from stage 3 to stage 5. This observation shows that serum Beta-Trace Protein increases as renal function declines and inversely correlated with Creatinine clearance (r = - 0.765).

Serum creatinine and blood urea were progressively increased in cases than controls and shows positive correlation with Beta-Trace Protein (r = 0.630 and r = 0.721) respectively.

Random Blood Sugar is significantly higher in cases(116 ± 39.8) than in controls (93.94 ± 6.97) and it shows strong positive correlation (r =0.700) with Beta-Trace Protein, indicates Chronic Kidney Disease is prevalent in Diabetes Mellitus.

Blood pressure were significantly higher in cases than controls which indicates Chronic Kidney Disease is more prevalent in hypertension.

Unlike serum creatinine, Beta-Trace Protein are unaffected by muscle mass composition.

Earlier studies have shown that Beta-Trace Protein is a more suitable marker of GFR because of less extra renal interferences[67,68].

Thus Serum Beta-Trace Protein is more precise and accurate marker than serum creatinine in detecting renal dysfunction.

CONCLUSION

- The present study demonstrated that serum Beta-Trace Protein levels are significantly increased in patients with Chronic Kidney Disease.
- This increase in serum Beta-Trace Protein level is progressive from stage 3 to 5, as renal function declines.
- It is more precise and accurate marker than serum creatinine in detecting renal dysfunction.
- Thus Beta-Trace Protein can be used as a novel biomarker in the diagnosis of Chronic Kidney Disease.

LIMITATIONS

- The sample size was small
- Calculation of Ccr is based on serum creatinine which is a crude marker for GFR estimation
- Other valuable relevant markers like Cystatin C are not included in the study.

SCOPE FOR FUTURE STUDY

- Calculating Beta-Trace Protein – based GFR equation for detecting renal dysfunction

- Beta-Trace Protein may have roles in GFR estimation, especially in conditions in which serum Cystatin C cannot be used, such as in neonates and pregnant females

BIBILIOGRAPHY

1. Thalquotra M, Pandey R, Singh J, Agrawal BK, Sodhi KS, Evaluation of thyroid profile in patients with chronic kidney disease. J Pharm Biomed Sci. 2014; 4:143-147.
2. Tangri N, Stevens LA, Griffith J. A predictive model of progression of chronic kidney disease to kidney failure. JAMA, 2011; 305 (15):1553-9.
3. Hunsicker LG: The consequences and costs of chronic kidney disease before ESRD. J Am SOC Nephrol 15(5): 2004: 1363-1364.
4. Amato AA, Santos. GM. Neves F. Thyroid hormone action in chronic kidney disease. Current opinion in endocrinology, Diabetes and Obesity. 2008:15:459-465.
5. Murray CJ, Lopez AD, Mathers CD, Stein C. The global burden of disease 2000 project: aims, methods and data sources. Global programme on evidence for health policy discussion. World Health organization. Paper no 36 November 2001 (revised).
6. Kronenberg F. Emerging risk factors and markers of chronic disease progression. Nat Rev Nephrol 2009; 5:677-89.
7. Kidney Disease: Improving Global Outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guidelines for the evaluation and management of chronic kidney disease. Kidney inter., Suppl.2013;3:1-150.

8. Maack T, Hyung Park C, Camargo MJF. Renal filtration, transport and metabolism of proteins. In: The Kidney; Physiology and pathophysiology, edited by Seldin DW, Giebish G. New York: Ravan, 1992, P.3005-3038.
9. Itoch Y. Enomoto H, Takagi K, Kawai T. Clinical usefulness of serum alpha 1- macroglobulin as a sensitive indicator for renal insufficiency. Nephron 33: 69-70, 1983.
10. Urade Y, Hayaishi O. Biochemical, structural, genetic, physical, and pathophysiological features of lipocalin-type prostaglandin D synthase. Biochin Biophys Acta 2000; 1482: 259 – 71.
11. Priem F, Althas H, Brinbaun M, Sinha P, Conradt HS, Jung K. β-Trace protein in serum: a new marker of glomerular filtration rate in the creatinine-blind range. Clinchem 1999: 45(4): 567-8.
12. Santosh Varughese, G.T.John et al, Pre-tertiary hospital care of patients with chronic kidney disease in India, Indian journal Med Res 126, July 2007, 28-33.
13. Douglas C.Eaton and John P. Pooler, Renal Functions. Basic processes, and Anatomy. In: Hershel Raff and Michael Levitzky, editors. Medical Physiology, A Systems Approach. New York: Tata McGraw-Hill; 2011 .p.397-406.
14. Modified from National Kidney Foundation. K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease: Evaluation, classification and stratification. Am J Kidney Dis 39: suppl 1, 2002. S1-S266.

15. Harold A. Franch, William C.McClellan, and William E.Mitch, Chronic Kidney Disease: Pathophysiology and Influence of Dietary Protein. In: Robert J. Alpern and Steven C. Hebert, editors. Seldin and Giebisch's The Kidney. 4th edition. Burlington: Elsevier; 2008. P. 2620-2634.
16. Kara L.Lynch, Alan H.B.Wu. Renal Function. In: Micheal L.Bishop, Edward P. Fody, and Larry E.Schoeff, editors. Clinical Chemistry. 7th edition. Philadelphia: Wolters Kluwer, Lippincott Williams & Wilkins; 2013.p.584-585, 576-577.
17. Suresh Chandra Dash, Sanjay K.Agarwal, Incidence of Chronic Kidney Disease in India, Advance Access publications 11 october 2005, 232-33.
18. Sreejith Parameswaran, Chronic Kidney Disease in India, Health Sciences 2012;1(2); JS001.
19. Agarwal SK, Srivastava RK, Chronic kidney disease in India: challenges and solutions, Nephron Clin Pract, 2009; 111(3):c197-203.
20. Rao & Pereira, Chronic kidney disease in India- a hidden epidemic, Indian Journal Med Res 126, July 2007, 6-9.
21. Kidney Function Tests. In: Dr. Nesar Ahmed, editor. Clinical Biochemistry. New York: Oxford University Press; 2011. P. 70-74,85-89.
22. Christopher M Florkowski, Janice SC Chew- Harris, Methods of Estimating GFR- Different Equations Including CKD-EPI; Clin Biochem Rev. 2011 May; 32(2): 75-79.

23. Levey AS, Greene T, Kusek JW, Beck GJ: A simplified equation to predict glomerular filtration rate from serum creatinine. J Am Soc Nephrol 11: 155A, 2000.
24. Mohsen El Kossi and Meguid El Nahas, Epidemiology and Pathophysiology of Chronic Kidney Disease: Natural History, Risk Factors, and Management & Iain C. Macdougall and Kai-Uwe Eckardt, Anemia in Chronic Kidney Disease. In: John Feehally, Jurgen Floege and Richard J Johnson, editors. Comprehensive Clinical Nephrology. 3rd edition. Philadelphia: Mosby Elsevier; 2007.p.813-821, 853-860.
25. Diuretics, Kidney Diseases & Red Blood Cells, Anemia and Polycythemia. In: John E. Hall. Guyton and Hall Textbook of Medical Physiology. 12th edition. Philadelphia: Saunders Elsevier; 2011.p.401-407.
26. Mark E. Williams and Robert C.Stanton, Diabetic Kidney Disease. In: Brian J.G.Pereira, Mohammed H.Sayegh, Peter G. Blake, Vaidyanathapuram S. Balakrishnan, Bertrand L.Jaber, Annamaria T. Kausz and ColmC. Magee, editors. Chronic Kidney Disease, Dialysis, and Transplantation. 2nd edition. Philadelphia: Saunders Elsevier; 2005.p.31-37.
27. Arrigo Schieppati, Roberto Pisoni and Giuseppe Remuzzi, Pathophysiology of Chronic Kidney Disease & Daniel E. Weiner and Mark J. Sarnak, Cardiac Function and Cardiovascular Disease in Chronic Kidney Disease & Jay.B.Wish, Hematologic Manifestations of Chronic Kidney Disease. In: Arthur Greenberg, Alfred K. Cheung, Thomas M.Coffman, Ronald J. Falk

and J.Charles Jennette, editors. Primer on Kidney Diseases. 5th edition. Philadelphia: Saunders Elsevier; 2009 .p.506-513.

28. Barry M.Brenner. Brenner & Rector's The Kidney. 8th edition. Philadelphia: Saunders Elsevier; 2008.p.1728-1737.
29. Dietary Metals, Iron, Micronutrients and Nutrition. In: Gerald Litwack. Human Biochemistry and Disease. Burlington: Elsevier; 2008.p.739-744.
30. Uzma Mehdi, Robert D. Toto, Anemia, Diabetes and Chronic Kidney Disease, Diabetes care, 2009, 32(7), 1320-1326.
31. Kidney Disease Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO Clinical Practice Guideline for the Diagnosis, Evaluation, Prevention and Management of Chronic Kidney Disease-Mineral and Bone Disorder(CKD-MBD). Kidney inter., Suppl. 2009; S1-130.
32. Carl A. Burtis, Edward R. Aswood, David E. Bruns: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics; 5th Edition. Saunders, Elsevier; p.1549-1551.
33. Dan L. Longo, Dennis L. Kasper, J. Larry jameson, Anthony S. Fauci, Stephen L. Hauser and Joseph Loscalzo, editors. Harrison's Principles of Internal Medicine. 19th edition. New York: McGraw- Hill; 2012. P.1811-1820.
34. Massy ZA, Nguyen Khoa T, Lacour B, Descamps-Latscha B, Man NK, Jungers P. Dyslipidaemia and the Progression of renal disease in chronic renal failure patients. Nephrol Dial Transplant 1999;14: 2392-7

35. Baigent C, Burbury K, Wheeler D. Premature cardiovascular disease in Chronic renal failure. Lancet 2000;356:147-52.
36. Wheeler DC, Townend JN, Landray MJ. Cardiovascular risk factors in predialysis patients: baseline data from the Chronic Renal Impairment in Birmingham (CRIB) Study. Kidney Int Suppl 2003; S201-3.
37. Menon V, Sarnak MJ: The epidemiology of Chronic kidney disease stages 1 to 4 and Cardiovascular disease: A high-risk combination. Am J Kidney Dis 45:223-232, 2005.
38. Silberberg JS, Barre PE, Prichard SS, Sniderman AD. Impact of left ventricular hypertrophy on survival in end stage renal disease. Kidney Int 1989; 36: 286-90.
39. Hegarty J, and Foley RN. Anemia, Renal insufficiency and Cardiovascular outcome. Nephrol Dial Transplant 2001;16: 102-104.
40. Rahman M, Xie D, Feldmen HI, et al. Association between Chronic kidney disease progression and Cardiovascular disease: results from the CRIC study. Am J Nephrol 2014;40: 399-407.
41. Luft FC. Renal disease as a risk factor for cardiovascular disease. Basic Res Cardiol. 2000; 95: 172-176.
42. Anavekar NS, Pfeffer MA. Cardiovascular risk in Chronic Kidney disease. Kidney Int 2004; 66: S11-S15.
43. Menon V, Gul A, Sarnak MJ: Cardiovascular risk factors in Chronic kidney disease. Kidney Int 68: 1413-1418, 2005.

44. Csaba P, Kovesdy and Kamyar Kalantar- Zadeh, Inflammation in Chronic Kidney disease. In: Jonathan Himmelfarb and Mohamed H.Sayegh, editors. Chronic kidney disease, Dialysis and Transplantation. 3rd edition. Philadelphia: Saunders Elsevier; 2010.p.183-197.
45. Clausen J. Proteins in normal cerebrospinal fluid not found in serum. Proc. Soc. Exp. Biol. Med. 107, 170–172 (1961).
46. White CA, Ghazan-Shahi S, Adams MA. β-Trace protein: a marker of GFR and other biological pathways. Am J Kidney Dis. 2015;65(1): 131–146.
47. Urade Y, Hayaishi O: Prostaglandin D synthase: Structure and function. Vitam Horm 58: 89–120, 2000.
48. Urade Y& Hayaishi O: Prostaglandin D_2 and sleep regulation. Biochem Biophysics Acta 1436, (1999); 606-615.
49. Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, Sugimoto Y, Kobayashi T, Ushikubi F, Aze Y et al. (2000). Prostaglandin D_2 as a mediator of allergic asthma. Science 287, 2013-2017.
50. Hayaishi O & Urade Y : Prostaglandin D_2 in sleep-wake regulation: recent progress and perspectives Neuroscientist 8, 2002; 12-15.
51. Hoffmann A, Conradt HS, Gross G, Nimtz M, Lottspeich F, Wurster U. Purification and chemical characterization of beta-trace protein from human cerebrospinal fluid: its identification as Prostaglandin D synthase. J Neurochem 61: 1993, 451-456.

52. Melegos DN, Grass L, Pierratos A, Diamandis E. Highly elevated levels of Prostaglandin D synthase in the serum of patients with renal failure. Urology 53: 1999, 32-37.
53. Andrea Hoffmann, Manfred Nimtz and Harald S. Condradt. Molecular characterization of Beta-trace protein in human serum and urine: a potential diagnostic marker for renal diseases. Glycobiology 7: 1997, 499-506.
54. Donadio C, Lucchesi A, Ardini M, Donadio E, Giordani R: Serum levels of beta-trace protein and glomerular filtration rate- Preliminary results. J Pharm Biomed Anal 32: 2003, 1099-1104.
55. Tariq shafi, Rulan S.Parakh, Bernard G.Jaar, Laura C.Plantinga, Pooja C.Oberai, John H. Eckfeldt, Andrew S.Levey, Neil R. Powe and Josef Coresh: Serum beta-trace protein and Risk of mortality in incident hemodialysis patients. Clin J Am Soc Nephrol 7: 2012, 1435–1445.
56. Beuckmann CT, Aoyagi M, Okazaki I, Hiroike T, Toh H, Hayaishi O, Urade Y: Binding of biliverdin, bilirubin and thyroid hormones to lipocalin-type prostaglandin D synthase. Biochemistry 38: 1999, 8006-8013.
57. Eguchi Y, Eguchi N, Oda H, Seiki K, Kijima Y, Matsu-Ura Y, Urade Y, Hayaishi O: Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart and its accumulation in the coronary circulation of angina pectoris. Proc Natl Acad Sci USA 94: 1997, 14689-14694.

58. Nagata N, Fujimori K, Okazaki I, Oda H, Eguchi N, Uehara Y, Urade Y: Denovo synthesis, uptake and proteolytic processing of lipocalin-type prostaglandin D synthase, beta-trace in the kidneys. FEBSJ 276: 2009, 7146-7158.
59. Bachmann- Harildstad G: Diagnostic values of beta-2 transferrin and beta-trace protein as markers for cerebrospinal fluid fistula. Rhinology, 2008 Jun; 46(2): 82-5.
60. Reiber H,. Walther K, Althaus H: Beta- trace protein as sensitive marker for CSF rhinorrhoea and CSF otorrhoea. Acta Neural Scand. 2003 Nov; 108(5): 359-62.
61. Orenes- Pinero E, Monzano- Fernandez S, Lopez- Cuenca A, Macin F, Valdes M, Januzzi JL: Beta-trace protein: from GFR marker to cardiovascular risk predictor. Clin J Am Soc Nephrol. 2013 May; 8(5): 873-81.
62. Hirawa N, Uehara Y, Yamakado M, Toya Y, Gomi T, Ikeda T, Eguchi Y, Takagi M, Oda H, Seiki K, Urade Y, Umamura S: Lipocalin-type prostaglandin D synthase in essential hypertension. 39: 2002, 449-454.
63. Uehara Y, Makino H, Seiki K, Urade Y; L-PGDS Clinical Research Group of kidney: Urinary excretions of lipocalin-type of prostaglandin D synthase predict renal injury in type-2 diabetes: A cross- sectional and prospective multicentre study. Nephrol Dial Transplant 24: 2009, 475-482.

64. Kanaaka Y, Urade Y. Hematopoietic prostaglandin synthase. Prostaglandins Leukot Essent Fatty acids 2003; 69: 163-167.
65. Chenn HH: Beta-trace protein versus cystatin C: Which is a better surrogate marker of renal function versus prognostic indicator in cardiovascular disease? J Am Coll Cardiol 57: 2011, 859-860.
66. Filler G, Lopes L, Harrold J, Bariciak E. Beta-trace protein may be a more suitable of neonatal renal function. Clin Nephrol. 2014 Apr;81(4):269-76.
67. Poge U, Gerhardt T, Stoffel-Wagner B, Palmedo H, Klehr HU, Sauerbruch T, Woitas RP: Beta-trace protein-based equations for calculation of GFR in renal transplant recipients. Am J Transplant 8: 2008, 608–615.
68. Bhavsar NA, Appel LJ, Kusek JW, Contreras G, Bakris G, Coresh J, Astor BC; AASK Study Group: Comparison of measured GFR, serum creatinine, cystatin C, and beta-trace protein to predict ESRD in African Americans with hypertensive CKD. Am J Kidney Dis 58: 2011, 886–893.

STUDY OF SERUM BETA-TRACE PROTEIN IN CHRONIC KIDNEY DISEASE –PROFORMA

NAME OF THE PATIENT :

AGE/SEX :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

GENERAL EXAMINATION:

Ht: Wt: BMI: BP: PR:

SYSTEMIC EXAMINATION:

CVS: RS:

ABD: CNS:

INVESTIGATIONS :

1.BLOOD UREA:

2.SERUM CREATININE

3. RANDOM BLOOD SUGAR:

4. SERUM BETA-TRACE PROTEIN:

CONSENT FORM

Dr. R.SUSILA post graduate student in the Department of Biochemistry, Thanjavur medical college, Thanjavur is doing a dissertation on Study of Beta-Trace Protein in Chronic Kidney Disease. The procedure has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature:

Name:

Place:

MASTER CHART I – CONTROL GROUP

S.NO	AGE (YRS)	SEX	HT (m)	WT (Kg)	BMI (kg/m^2)	SBP (mm/Hg)	DBP (mm/Hg)	BTP (ng/ml)	CREAT (mg/dl)	Ccr (ml/min)	UREA (mg/dl)	RBS (mg/dl)
1	54	M	1.6	64	24	122	82	33	0.7	114.2	33	96
2	51	F	1.5	50	22	110	70	23	0.8	101.9	23	93
3	54	M	1.65	70	25	100	80	21	0.9	118.31	36	91
4	61	M	1.53	60	25	120	80	10	0.8	133.7	32	90
5	58	M	1.65	65	23	110	80	13	0.9	117.36	35	98
6	54	M	1.48	55	25	100	82	12	0.9	117.9	38	92
7	49	F	1.48	60	27	122	70	21	1	112.22	42	98
8	57	F	1.45	63	29	123	72	23	0.7	109.6	27	96
9	48	F	1.55	60	24	130	76	23	0.8	117.5	32	89
10	49	M	1.54	62	26	100	80	23	0.9	105.57	40	90
11	61	M	1.5	55	24	120	80	23	0.7	121.7	36	92
12	60	F	1.52	58	25	114	74	12	0.7	129.51	32	94
13	54	F	1.55	65	27	110	70	32	0.8	95.08	38	98
14	58	F	1.6	55	21	130	80	23	0.7	119.5	34	86
15	52	F	1.52	60	25	110	80	32	0.6	125.35	37	92
16	53	F	1.53	60	25	126	84	27	0.9	115.08	27	96
17	54	M	1.52	58	25	124	80	30	0.9	116.19	42	90
18	58	M	1.48	55	25	110	80	30	0.8	110.78	40	96
19	54	M	1.6	55	21	128	80	32	0.9	114.3	32	88
20	49	M	1.67	72	25	122	80	34	1	125.1	40	98
21	51	M	1.65	65	23	126	82	34	0.9	112.5	34	86
22	52	M	1.67	60	21	120	80	30	0.8	118.61	39	84

23	57	M	1.7	70	24	112	74	30	0.7	112.34	36	90
24	55	F	1.6	75	29	130	80	32	0.9	121.55	44	80
25	54	M	1.6	70	27	120	80	32	1	101.19	32	98
26	49	F	1.68	70	24	128	74	23	0.9	113.33	39	90
27	45	M	1.7	80	27	110	70	11	1.1	123.15	40	102
28	51	M	1.65	69	25	120	80	44	1.1	106.94	42	104
29	48	M	1.68	75	26	116	84	38	0.9	111.63	38	98
30	52	F	1.72	75	25	110	72	29	1.1	103.11	39	84
31	43	F	1.69	72	25	110	70	39	0.9	117.5	39	96
32	54	M	1.65	70	25	122	82	50	1	128.68	41	102
33	52	F	1.6	69	26	110	80	43	0.8	115.5	42	92
34	51	F	1.67	70	25	126	80	50	1.1	104.4	43	82
35	58	M	1.71	75	25	120	82	32	0.6	102.84	32	106
36	46	F	1.6	64	24	126	84	46	1	122.52	41	106
37	49	F	1.68	74	26	110	80	46	0.9	121.9	42	104
38	43	F	1.65	70	25	114	78	49	1.1	110.59	43	102
39	45	M	1.68	75	26	130	80	48	0.8	106.7	35	86
40	48	F	1.69	74	25	110	70	50	0.9	117.36	38	90
41	52	F	1.67	67	24	116	84	45	1	116.9	40	104
42	46	M	1.68	70	24	120	80	43	0.9	125	43	84
43	49	F	1.67	67	24	110	70	49	1.1	104.46	41	98
44	47	M	1.7	71	24	120	80	50	0.8	112.59	34	102
45	45	F	1.67	72	25	120	80	49	0.9	129.6	43	82
46	52	M	1.7	80	27	126	82	45	0.9	126.3	44	102
47	51	F	1.72	65	21	120	80	50	1.1	117.8	41	92
48	54	F	1.69	72	25	118	76	32	1	103.8	32	104
49	51	F	1.62	68	25	120	80	49	0.9	104.27	42	98
50	56	M	1.68	74	26	110	70	48	1.1	122.6	40	86

MASTER CHART II – STUDY GROUP

S.NO	AGE (YRS)	SEX	HT (m)	WT (Kg)	BMI (kg/m^2)	SBP (mm/Hg)	DBP (mm/Hg)	BTP (ng/ml)	CREAT (mg/dl)	Ccr (ml/min)	UREA (mg/dl)	RBS (mg/dl)
1	46	M	1.5	55	24	100	70	80	2.1	34.19	65	86
2	49	F	1.56	64	26	150	100	32	1.7	40.44	30	98
3	47	F	1.55	60	24	110	80	76	2.4	27.7	75	108
4	45	F	1.52	65	28	100	80	30	1.5	49.13	31	118
5	52	M	1.55	56	23	110	70	30	1.8	38	32	98
6	51	F	1.6	60	23	140	100	64	1.8	35.02	62	220
7	54	F	1.6	79	30	120	80	66	2.5	29.24	161	104
8	51	F	1.65	73	26	100	80	66	1.7	45.12	75	98
9	56	F	1.72	74	25	160	100	74	2.2	33.35	72	210
10	54	M	1.68	70	24	110	70	63	1.7	49.18	58	96
11	58	F	1.64	78	29	170	110	34	1.5	50.34	33	90
12	52	F	1.67	78	27	90	70	86	1.9	42.6	64	96
13	53	M	1.7	71	24	100	80	32	1.8	47.66	32	114
14	54	F	1.6	68	26	110	70	65	2.3	30.01	141	92
15	58	M	1.68	64	22	100	80	66	1.7	42.87	46	108
16	54	M	1.7	69	23	140	90	23	1.6	55.98	35	98
17	49	F	1.65	70	25	100	80	54	2.4	31.33	50	84
18	51	M	1.62	70	26	170	110	62	1.8	48.07	59	176
19	52	F	1.6	78	30	110	80	25	1.8	45.01	33	94
20	46	M	1.8	56	17	142	90	23	1.9	38.47	35	112
21	49	F	1.8	68	20	142	90	149	18.9	4.55	179	92
22	43	F	1.64	72	26	142	80	23	1.8	45.8	33	104
23	45	F	1.67	83	29	142	80	32	1.8	51.7	36	210
24	48	M	1.78	75	23	100	70	21	1.5	63.88	78	108

25	52	F	1.78	70	22	152	94	64	1.6	45.45	76	98
26	46	F	1.64	86	31	180	110	41	1.8	53.29	82	88
27	52	F	1.7	82	28	120	80	80	3.5	24.33	175	92
28	53	M	1.71	70	23	110	70	70	1.6	52.86	58	86
29	54	F	1.6	72	28	100	80	72	1.7	42.99	47	94
30	58	M	1.7	78	26	110	90	36	1.9	46.75	38	78
31	54	F	1.67	78	27	120	80	34	1.7	46.58	35	96
32	49	M	1.72	76	25	144	96	32	1.6	60.03	34	102
33	51	M	1.7	73	25	110	80	26	1.5	60.15	48	188
34	52	M	1.7	70	24	120	80	64	4.3	19.89	52	90
35	51	M	1.87	64	18	134	70	63	1.8	43.95	55	120
36	52	F	1.7	70	24	132	80	24	1.6	45.44	47	118
37	57	M	1.6	65	25	110	80	23	1.7	44.07	46	92
38	55	M	1.65	70	25	100	70	26	1.6	51.65	28	104
39	54	F	1.7	70	24	120	70	80	1.5	47.38	47	112
40	49	M	1.64	70	26	100	70	23	1.7	52.04	28	78
41	45	M	1.72	76	25	156	80	63	1.9	52.77	52	192
42	51	F	1.7	78	26	156	80	32	1.5	54.64	32	94
43	48	M	1.68	75	26	176	90	12	1.8	53.2	30	108
44	52	M	1.64	70	26	168	100	145	6	14.26	160	118
45	43	M	1.72	60	20	120	80	94	2.6	31.08	47	116
46	49	M	1.6	65	25	168	90	82	2.8	29.34	140	96
47	57	F	1.69	80	28	156	88	33	1.5	52.26	33	88
48	48	M	1.7	82	28	158	80	22	1.6	65.48	32	76
49	49	M	1.72	79	26	168	90	32	1.5	66.56	30	92
50	61	F	1.64	78	29	156	90	63	2.4	30.3	68	104